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CONTENTS OF VOLUME VI

PART I (FEBRUARY 1936)

ORIGINAL ARTICLES—

		PAGE
CYTO-GENETIC ANALYSIS OF *Saccharum spontaneum* L., I.—CHROMOSOME STUDIES IN SOME INDIAN FORMS (WITH PLATES I-V).	*E. K. Janaki Ammal* .	1
CYTO-GENETIC ANALYSIS OF *Saccharum spontaneum* L., 2.—A TYPE FROM BURMA (WITH PLATES VI-VIII).	*E. K. Janaki Ammal and T. S. N. Singh, B.Sc.*	9
SOME NEW SEEDLING DISEASES OF SUGARCANE (WITH PLATES IX-XI).	*L. S. Subramaniam* .	11
A BIOCHEMICAL STUDY ON THE DECOMPOSITION OF FARMYARD MANURE AND SULPHATE OF AMMONIA IN THE PADDY SOILS OF THE CENTRAL PROVINCES.	*B. K. Mukerji, Ph.D., D.Sc. and S. L. Vishwoi, B.Ag.*	17
STATISTICAL NOTES FOR AGRICULTURAL WORKERS, NO. 16.—A COMPLEX EXPERIMENT ON RICE AT THE CHINSURAH FARM, BENGAL, 1933-34.	*Suresh Chandra Chakravertti, Subhendu Sekhar Bose and P. C. Mahalanobis*	34
INFLUENCE OF STORAGE ON SOME OF THE FIBRE-PROPERTIES OF DHARWAR COTTON (WITH TWO TEXT-FIGURES).	*H. R. Nayak* . .	52
VARIATION IN THE FIBRE-PROPERTIES OF SIMILAR STRAINS OF COTTON GROWN IN DIFFERENT TRACTS OF GUJARAT (WITH ONE TEXT-FIGURE).	*B. S. Patel, I. A. S. and Srinagabhushana, B.Sc.*	63
DISEASES OF *Citrus* IN THE PUNJAB (WITH PLATES XII-XIX AND ONE TEXT-FIGURE).	*H. Chaudhuri* . .	72
A NEW VARIETY OF *Oidiopsis taurica* (WITH PLATE XX AND ONE TEXT-FIGURE).	*B. N. Uppal, Ph.D.; M. N. Kamat, M.Sc. and M. K. Patel, Ph.D.*	110
MICROSPOROGENESIS IN *Crotalaria juncea* LINN. (WITH PLATES XXI AND XXII).	*I. Banerji and K. K. Samal*	116

RESEARCH NOTE—

EFFECTS OF BROWN RUST ATTACK ON WHEAT	*B. P. Pal., M.Sc., Ph.D., F.L.S.*	127

NOTICE—

	PAGE
BIBLIOGRAPHY OF SOIL SCIENCE, FERTILIZERS AND GENERAL AGRONOMY.	129

PART II (APRIL 1936)

ORIGINAL ARTICLES—

STUDIES IN INDIAN PULSES: THE INHERITANCE OF MORPHOLOGICAL CHARACTERS AND OF WILT RESISTANCE IN *Rahar* (*Cajanus indicus* SPRENG.) (WITH PLATES XXIII—XXVI).	*F. J. F. Shaw, D.Sc. (Lond.) A.R.C.S., F.L.S., I.A.S.*	139
STUDIES ON *Schistocerca gregaria* FORSK., II. THE BIOLOGY OF THE DESERT LOCUST WITH SPECIAL RELATION TO TEMPERATURE (WITH SIX TEXT-FIGURES).	*M. Afzal Husain, M.A. (Cantab.), I. A. S., and Teshkir Ahmad, B.Sc. (Agri.)*	188
STUDIES ON *Schistocerca gregaria* FORSK., III. WHY LOCUSTS EAT WOOL: A STUDY IN THE HYDROMANIA OF *Schistocerca gregaria*.	*M. Afzal Husain, M.A. (Cantab.), I.A.S. and C. B. Mathur, M.Sc.*	263
NITROGEN RECUPERATION IN THE SOILS OF THE BOMBAY PRESIDENCY, PART IV (WITH THIRTEEN TEXT-FIGURES).	*D. L. Sahasrabuddhe, M.Ag., M.Sc. and V. S. Abhyankar, B.Ag.*	268
NITROGEN BALANCE IN BLACK COTTON SOILS IN THE MALWA PLATEAU, II. CHANGES DURING THE HOT WEATHER (WITH THREE TEXT-FIGURES).	*Yeshwant D. Wad and R. K. Aurangabadkar*	316
STUDIES IN INDIAN FIBRE PLANTS, NO. 4. THE ROOT-SYSTEM OF SUNN-HEMP (*Crotalaria juncea* L.) (WITH PLATES XXVII AND XXVIII).	*R. D. Bose, B.Sc.; M.A. Aziz, B.Sc. (Agri.) and M. P. Bhatnagar, B.Sc. (Hons.)*	351
EARLINESS IN THE UNITED PROVINCES RICES (WITH TWO TEXT-FIGURES).	*R. L. Sethi, M.Sc., B.Sc. (Edin.), I.A.S.; B. L. Sethi, M.Sc., Ph.D. (Wales) and T. R. Mehta, B.Sc.*	361
A PRELIMINARY NOTE ON COTTON FAILURE IN THE PUNJAB AND SOME ABNORMALITIES IN THE PLANT (WITH PLATES XXIX—XXXI).	*R. H. Dastur . .*	377
DEVELOPMENTAL VARIATION IN THE PADDY GRAIN.	*U Tin (1), Dip. Agri.*	396
STATISTICAL NOTES FOR AGRICULTURAL WORKERS, NO. 17.—ON THE INFLUENCE OF SHAPE AND SIZE OF PLOTS ON THE EFFECTIVE PRECISION OF FIELD EXPERIMENTS WITH *Juar* (*Andropogon Sorghum*).	*R. K. Kulkarni, B.Ag.; Subhendu Sekhar Bose and P. C. Mahalanobis*	460

		PAGE
PRELIMINARY OBSERVATIONS ON SMALL-LEAF DISEASE IN COTTON.	*V. P. Gokhale, B.Ag.* .	475
INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET, VIII. A BROWNISH PURPLE MUTANT (WITH PLATE XXXII).	*G. N. Rangaswami Ayyangar, B.A., I.A.S. and A. Kunhikoran Nambiar, B.Sc. Ag.*	481
BANANA BREEDING AT THE IMPERIAL COLLEGE OF TROPICAL AGRICULTURE, TRINIDAD, WITH A VIEW TO OBTAIN A TYPE RESISTANT TO THE PANAMA DISEASE, INCLUDING GENETICAL AND CYTOLOGICAL STUDIES ON ALLIED SPECIES.	*G. S. Cheema, D.Sc., I.A.S. and S. S. Bhat, M.Ag.*	484
ABSTRACT		502
NOTE—		
RESOLUTIONS CARRIED BY THE SIXTH INTERNATIONAL BOTANICAL CONGRESS.		504

PART III (JUNE 1936)

ORIGINAL ARTICLES—		
ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY. PART IV. INTERPRETATION OF TITRATION CURVES (WITH TWENTY TEXT-FIGURES).	*Jnanendranath Mukherjee, D. Sc.; Ramprasad Mitra, M.Sc.; Santosh Chandra Ganguli, M.Sc. and Baradananda Chatterjee, M.Sc.*	517
ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY, PART V. TITRATION CURVES OF CLAY ACIDS (WITH ELEVEN TEXT-FIGURES)	*Ramprasad Mitra, M.Sc.*	555
INDIAN SOIL FUNGI	*L. D. Galloway* . .	578
STUDIES ON *Schistocerca gregaria* FORSK., IV. COLOUR CHANGES AND SEXUAL BEHAVIOUR IN DESEXUALISED *Schistocerca gregaria* ADULTS.	*M. Afzal Husain, M.A. (Cantab.), I.A.S. and Kesho Das Baweja, M.Sc.*	586
STUDIES ON *Schistocerca gregaria* FORSK., V. PIGMENTATION AND PHYSICAL EXERTION (WITH PLATES XXXIII—XXXV).	*M. Afzal Husain, M.A. (Cantab.), I.A.S. and C. B. Mathur, M.Sc.*	591
STUDIES ON *Schistocerca gregaria* FORSK., VI. INFLUENCE OF TEMPERATURE ON THE INTENSITY AND EXTENT OF BLACK PATTERN IN THE DESERT LOCUST HOPPERS BRED CROWDED (WITH PLATES XXXVI—XL.)	*M. Afzal Husain, M.A. (Cantab.), I.A.S. and Taskhir Ahmad, B.Sc. (Agri.)*	624

		Page
Studies on *Schistocerca gregaria* Forsk., VII. Factors determining the Movement of the Vermiform Larvae (with four text-figures).	*M. Afzal Husain, M.A. (Cantab.), I.A.S. and Des Raj Bhatia, M.Sc.*	665
Studies in Plant Breeding Technique, I. An Analysis of the Efficiency of Selection Methods used in the Improvement of Malvi Cotton.	*J. B. Hutchinson and Kubersingh*	672
Ideal Size and Shape of Sugarcane Experimental Plots based upon Tonnage Experiments with Co 205 and Co 213 conducted in Pusa (with Plate XLI and two text-figures)	*Wynne Sayer, B.A., Dip. Agri. (Cantab.); M. Vaidyanathan, M.A., L.T. and S. Subramonia Iyer, M.A.*	684
The Basis for Merging *Sclerospora indica* with *Scl. philippinensis* (with Plate XLII).	*B. N. Uppal and Wm. H. Weston, Jr.*	715
The Tolerance of Wheat Plants for Chlorides of certain Non-Essential Elements (with eight text-figures).	*B. N. Singh, D.Sc. and S. Prasad, M.Sc.*	720
A Biochemical Study of the Paddy Soils of Bengal with special reference to their Nitrogen-fixing Capacities.	*Pran Kumar De and Anil Kumar Pain*	746
Bismuthate Method for the Estimation of Manganese in Plant Material.	*Ramji Narain, Ph.D., D.Sc. and Azmat Singh, L.Ag.*	757
Utilization of certain Forms of Inorganic Nitrogen during the Decomposition of Plant Materials in the Soil	*Jageshwar Gopal Shrikhande, M.Sc., Ph.D., A.I.C.*	767
On the Nature of Nitrification in Soil .	*S. V. Desai, B.Sc., Ph.D., D.Sc., D.I.C. and Fazal-ud-Din, B.Sc.*	777
Studies in the Germination of Crop Seeds in the Field (with two text-figures.)	*Labh Singh, B.Sc. (Agri.), L.Ag. and Nek Alam, B.Sc. (Agri.)*	784
A Phoma Disease of Asparagus (with Plate XLIII).	*K. F. Kheswalla* .	800
Gummosis of Citrus in Bombay (with Plates XLIV—XLVI and two text-figures).	*B. N. Uppal and M. N. Kamat*	803
A Note on the Hairiness of Cotton . .	*Mohammad Afzal, B.Sc. (Agri.), A.I.C.T.A.*	823

		PAGE
A NOTE ON THE STERILE HYBRID BETWEEN *Nicotiana Tabacum* L. and *N. plumbaginifolia* VIV. (WITH PLATE XLVII AND ONE TEXT-FIGURE).	*B. P. Pal, M.Sc., Ph.D. (Cantab.), F. L. S., and Pushkar Nath, M.Sc. (Hons.)*	828
A DISEASE OF *Zea Mays* CAUSED BY *Colletotrichum graminicolum* (CES) WILS (WITH PLATES XLVIII AND XLIX AND ONE TEXT-FIGURE).	*S. C. Chowdhury* .	833
REDUCTION OF NITRATES BY THE SUN . .	*Fazal-ud-Din, B.Sc.* .	844
INHERITANCE OF SOME MORPHOLOGICAL CHARACTERS IN *Crepis dioscoridis*.	*Swarn Singh Purewal, M.Sc. (Calif.), Ph.D. (Cornell.).*	855
A NOTE ON A NEW TYPE OF PROGRESSIVE DAMAGE TO THE STRUCTURE OF COTTON HAIR CAUSED BY MICRO-ORGANISMS (WITH PLATES L AND LI AND ONE TEXT-FIGURE).	*A. N. Gulati, M.Sc.* .	861
SELECTED ARTICLE—		
METHODS OF ANALYSIS AND APPRECIATION OF WHEAT, FLOURS AND BREAD, PARTICULARLY IN RESPECT OF INVESTIGATIONS ON THE BAKING VALUE (WITH PLATE LII).	*Prof. Dr. Luigi Borasio*	866
MYCOLOGICAL NOTE—		
ON THE SYSTEMATIC POSITION OF THE SMUT CAUSING MALFORMATION OF *Vitis quadrangularis*.	*B. B. Mundkur* . .	876
NOTES—		
TABLES OF STANDARD ERRORS OF MENDELIAN RATIOS.	..	878
PROCLAMATION OF THE UNION OF SOUTH AFRICA GOVERNMENT.	..	878
REVIEW—		
VERNALIZATION AND PHASIC DEVELOPMENT OF PLANTS.	..	882

PART IV (AUGUST 1936)

ORIGINAL ARTICLES—		
STUDIES ON *Bemisia gossypiperda* M. and L., No. 3. SEASONAL ACTIVITIES OF *Bemisia gossypiperda* M. AND L. (THE WHITE-FLY OF COTTON) IN THE PUNJAB (WITH TWO TEXT-FIGURES).	*M. Afzal Husain, M.A. (Cantab.); Kidar Nath Trehan, M.Sc. and Piare Mohan Verma, M.Sc.*	893

		PAGE
✓ STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB, II. SOME STUDIES IN THE PHYSIOLOGY OF THE CAUSAL FUNGI (WITH TWO TEXT-FIGURES).	*R. Sahai Vasudeva, B.Sc., Ph.D. (Lond.), D.I.C.*	904
ON SOME OF THE FACTORS THAT INFLUENCE THE ERROR OF FIELD EXPERIMENTS WITH SPECIAL REFERENCE TO SUGARCANE.	*Wynne Sayer, B.A., Dip. Agri. (Cantab.) and P. V. Krishna Iyer, M.A.*	917
THE CLASSIFICATION OF THE AUTUMN RICES OF THE PUNJAB AND WESTERN UNITED PROVINCES.	*Kashi Ram and R. B. Ekbote, L. Ag. (Hons.)*	930
INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET, IX. DIMPLED GRAINS (WITH PLATE LIII AND ONE TEXT-FIGURE).	*G. N. Rangaswami, Ayyangar, B.A., I.A.S.; M.A. Sankara Ayyar, B.A., B.Sc. Ag.; V. Panduranga Rao, M.A. and A. Kunhikoran Nambiar, B.Sc.Ag.*	938
THE STORAGE OF FUNGAL CULTURES (WITH ONE TEXT-FIGURE).	*L. D. Galloway* . .	946
BASE EXCHANGE STUDIES, I. A PRELIMINARY STUDY OF THE EFFECT OF CERTAIN CATIONS SATURATING THE SOIL'S EXCHANGE COMPLEX, ON ITS PHYSICO-CHEMICAL PROPERTIES AND THEIR RELATION TO THE PLANT GROWTH.	*Dalip Singh, M.Sc. (Punjab), Ph.D. (Cantab.) and Sukh Dayal Nijawan, M.Sc.*	956
SOME STUDIES ON THE SCLEROTIAL DISEASES OF RICE (*Sclerotium oryzae* CATT.) IN THE PUNJAB (WITH PLATE LIV).	*Jai Chand Luthra, M.Sc., D.I.C. (Lond.), I.A.S. and Abdus Sattar, B.Sc. (Ag.), Ph.D. (Lond.), D.I.C. (Lond.)*	973
PHOTO-CHEMICAL OXIDATION BY PLANT MATERIALS.	*S. V. Desai, D.Sc., Ph.D., D.I.C. and Fazal-ud-Din., B.Sc.*	985
ABSTRACT		991
MYCOLOGICAL NOTES—		
A DISEASE OF *Cyperus tegetiformis* ROXB. CAUSED BY *Kawakamia cyperi* (MIYABE AND IDETA) MIYABE (WITH PLATE LV)	*C. B. Sahaya* . .	992
A RHIZOCTONIA ON SWEET POTATOES IN BOMBAY (WITH PLATE LVI).	*B. B. Mundkur* . .	994
REVIEW—		
A COMPARATIVE STUDY OF CERTAIN MORPHOLOGICAL CHARACTERS OF SUGARCANE × SORGO HYBRIDS.	. .	996

PART V (OCTOBER 1936)

F. J. F. SHAW, C.I.E., D.Sc., A.R.C.S., F.L.S. (Obituary)

ORIGINAL ARTICLES—

		PAGE
STUDIES ON *Schistocerca gregaria* FORSK. VIII. INFLUENCE OF CARBON DIOXIDE ON DEVELOPMENT OF BLACK PIGMENTATION IN *Schistocerca gregaria* FORSK. (WITH PLATES LVII-LIX).	*M. Afzal Husain, M.A. (Cantab.) and C. B. Mathur, M.Sc. (Luck.)*	1005
THE LOCUST INCURSION OF 1935 IN NORTH-WEST INDIA: ITS SIGNIFICANCE IN THE STUDY OF THE LOCUST PROBLEM (WITH TWO MAPS AND ONE TEXT-FIGURE).	*Y. Ramchandra Rao, M.A.*	1031
SINGLE-VALUE SOIL PROPERTIES: MOISTURE AT THE STICKY POINT AND *R*.	*J. Charlton, M.Sc., F.I.C., I.A.S.*	1054
RAPID DETERMINATION OF SOIL-MOISTURE BY DRYING THE SOIL OVER BUNSEN BURNER.	*Ashutosh Sen* . .	1076
STUDIES ON THE ORGANIC NITROGEN OF PADDY SOILS, PART I. DISTRIBUTION OF HUMIC AND NON-HUMIC NITROGEN IN ORGANIC MATTER.	*Pran Kumar De and Anil Kumar Pain*	1081
THE INHERITANCE OF SEED-CHARACTERS IN GRAM (*Cicer arietinum* L.) (WITH PLATES LX AND AND LXI)	*Hukum Singh, B.Sc. (Agri.) and R. B. Ekbote, L. Ag. (Hons.)*	1087
A PRELIMINARY NOTE ON A NEW SACCHARUM × SORGHUM HYBRID (WITH PLATES LXII AND LXIII)	*E. K. Janaki Ammal and T. S. N. Singh*	1105
STATISTICAL NOTES FOR AGRICULTURAL WORKERS—		
No. 19.—THE FREQUENCY DISTRIBUTION OF PLOT YIELDS AND THE OPTIMUM SIZE OF PLOTS IN A UNIFORMITY TRIAL WITH RICE IN ASSAM (WITH THREE TEXT-FIGURES).	*S. S. Bose, P. M. Ganguli and P. C. Mahalanobis*	1107
No. 20.—STUDIES IN TILLER VARIATION (WITH THREE TEXT-FIGURES).	*K. C. Banerjee, S. S. Bose and P. C. Mahalanobis*	1122

SELECTED ARTICLE—

METHODS OF ANALYSIS AND APPRECIATION OF WHEAT, FLOURS AND BREAD, PARTICULARLY IN RESPECT OF INVESTIGATIONS ON THE BAKING VALUE, II (WITH TWO TEXT-FIGURES)	*Prof. Dr. Luigi Borasio*	1134
ABSTRACT (WITH PLATES LXIV AND LXV)	..	1153
REVIEW—		
PLANT BREEDING ABSTRACTS SUPPLEMENT II	..	1155

PART VI (DECEMBER 1936)

ORIGINAL ARTICLES—

		PAGE
AGRICULTURAL METEOROLOGY : STUDIES ON SOIL-MOISTURE IN RELATION TO MOISTURE IN THE SURFACE LAYERS OF THE ATMOSPHERE DURING THE CLEAR SEASON AT POONA (WITH PLATE LXVI AND TEN TEXT-FIGURES).	*L. A. Ramdas, M.A., Ph.D. and M. S. Katti, B.Sc.*	1163
STUDIES ON SOIL-MOISTURE, I. MOVEMENT OF SOIL-MOISTURE UNDER FIELD CONDITIONS (WITH EIGHT TEXT-FIGURES).	*M. Carbery, D.S.O., M.C., M.A., B.Sc. and M. N. Chakladar, M.Sc.*	1201
MINERAL MATTER IN THE JUICE OF SUGARCANE AND ITS EFFECT ON THE RECOVERY OF WHITE SUGAR, I.	*P. E. Lander, M.A., D.Sc., F.I.C. and Ramji Narain, Ph.D., D.Sc.*	1218
THE PROBLEM OF THE NITROGEN SUPPLY OF RICE, I. FIXATION OF NITROGEN IN THE RICE SOILS UNDER WATER-LOGGED CONDITIONS.	*Pran Kumar De* .	1237
INHERITANCE OF EARLINESS IN UNITED PROVINCES RICES, I (WITH FOUR TEXT-FIGURES).	*R. L. Sethi, M.Sc. (Pb.), B.Sc. (Agri.-Edin.), I.A.S.; B. L. Sethi, M.Sc., Ph.D. (Wales) and T. R. Mehta, B.Sc.*	1246
MENDELIAN SEGREGATION IN *Hibiscus sabdariffa* L. IN RELATION TO THE AGE OF THE F_1 AT TIME OF FERTILIZATION (WITH SEVEN TEXT-FIGURES).	*R. B. Deshpande, M. Ag. (Bom.)*	1274
AN ILLUSTRATION OF THE USE OF THE χ^2-TECHNIQUE ANALYSIS ON METEOROLOGICAL DATA.	*P. V. Sukhatme, B.Sc., Ph.D. (Lond.)*	1292
STUDIES IN SORGHUM—THE GREAT MILLET, III. ANTHER, POLLEN AND STIGMA (WITH PLATES LXVII AND LXVIII AND TWO TEXT-FIGURES)	*G. N. Rangaswami Ayyangar, B.A., I.A.S. and V. Panduranga Rao, M.A.*	1299
BLIGHT AND HOLLOW-STEM OF SORGHUM (WITH PLATES LXIX—LXXII AND ONE TEXT-FIGURE).	*B. N. Uppal, K. G. Kolhatkar and M. K. Patel*	1323

REVIEWS—

FLOUR MILLING PROCESSES . . .	..	1335
THE CULTIVATED RACES OF SORGHUM .	..	1335
DESTRUCTIVE INSECTS AND PESTS ACT, 1914	..	1337

INDEX TO VOLUME VI

A

PAGE

ABHYANKAR, V. S. (SAHASRABUDDHE, D. L. and)—Nitrogen recuperation in the soils of the Bombay Presidency 268

Abnormalities in the cotton plant (DASTUR, R. H.) 377

Acidity of soil, reactions responsible for (MUKHERJEE, J. N. *et al.*) . . 517

——————————————— (MITRA, RAMPRASAD) . . 555

Acids of clay, titration curves of (MITRA, RAMPRASAD) 555

Acrothecium lunatum, causing saprophytic moulds in citrus (CHAUDHURI, H.) 96

AFZAL HUSAIN, M. and AHMAD, TASKHIR—"Studies on *Schistocerca gregaria* Forsk., II. The Biology of the Desert Locust with special relation to Temperature" 188

——————————————— —"Studies on *Schistocerca gregaria* Forsk., VI. Influence of Temperature on the Intensity and Extent of Black Pattern in the Desert Locust Hoppers Bred Crowded" 624

——————— and BAWEJA, KESHO DAS—"Studies on *Schistocerca gregaria* Forsk., IV. Colour Changes and Sexual Behaviour in Desexualised *Schistocerca gregaria* Adults" 586

——————————— and BHATIA, DES RAJ—"Studies on *Schistocerca gregaria* Forsk., VII. Factors determining the Movement of the Vermiform Larvæ" 665

——————— and MATHUR, C. B.—"Studies on *Schistocerca gregaria* Forsk., III. Why Locusts Eat Wool: A Study in the Hydromania of *Schistocerca gregaria*" 263

——————————————— "Studies on *Schistocerca gregaria* Forsk., V. Pigmentation and Physical Exertion" . . 591

——————————————— "Studies on *Schistocerca gregaria* Forsk., VIII. Influence of Carbon Dioxide on Development of Black Pigmentation in *Schistocerca gregaria* Forsk." . 1005

——————; TREHAN, K. N. and VERMA, P. M.—"Studies on *Bemisia gossypiperda* M. & L. No. 3. Seasonal Activities of *Bemisia gossypiperda* M. & L. (the White-fly of Cotton) in the Punjab" 893

AFZAL, MOHAMMAD—"A Note on the Hairiness of Cotton" . . 823

Agricultural Meteorology (RAMDAS, L. A. and KATTI, M. S.) . . . 1163

PAGE

AHMAD, N. (GULATI, A. N. and)—Fibre-maturity of Indian cottons . 502

AHMAD, TASKHIR (AFZAL HUSAIN, M. and)—Studies on *Schistocerca gregaria* 188, 624

ALAM, NEK (LABH SINGH and)—Germination of crop seeds in the field . 784

Alternaria citri, causing saprophytic moulds in citrus (CHAUDHURI, H.) . 97

Ammonium sulphate, decomposition of, in paddy soils (MUKERJI, B. K. and VISHNOI, S. L.) 17

Andropogon Sorghum (sorghum), inheritance of characters in (RANGASWAMI AYYANGAR, G. N. *et al.*) 938

——————— (*juar*), influence of shape and size of plots on the effective precision of field experiments with (KULKARNI, R. K. *et al.*) 460

——————— (sorghum), anther, pollen and stigma in (RANGASWAMI AYYANGAR, G. N. and PANDURANGA RAO, V.) . . 1299

——————— (sorghum), blight and hollow-stem of (UPPAL, B. N. *et al.*) 1323

Anther in sorghum (RANGASWAMI AYYANGAR, G. N. and PANDURANGA RAO, V.) 1299

Asparagus, a phoma disease of (KHESWALLA, K. F.) 800

Aspidiotus orientalis, note on the *Chalcidoid* parasites of (GLOVER, P. M.). 991

Atmosphere, moisture in the surface layers of, in relation to soil moisture (RAMDAS, L. A. and KATTI, M. S.) 1163

AURANGABADKAR, R. K. (WAD, Y. D. and)—Nitrogen balance in black cotton soils in the Malwa Plateau 316

AZIZ, M. A. (BOSE, R. D. and)—Studies in Indian fibre plants . . 351

AZMAT SINGH (RAMJI NARAIN and)—Bismuthate method for the estimation of manganese in plant material. 757

B

Baking value, methods of analysis and appreciation of wheat, flours and bread, in respect of (BORASIO, LUIGI) 866, 1134

Banana breeding at the Imperial College of Tropical Agriculture (CHEEMA, G. S. and BHAT, S. S.) 484

BANERJI, I. and SAMAL, K. K.—"Microsporogenesis in *Crotalaria juncea* Linn." 116

BANERJEE, K. C.; BOSE, S. S. and MAHALANOBIS, P. C.—"Statistical Notes for Agricultural Workers, No. 20—Studies in Tiller Variation" 1122

Base-exchange studies in soils (DALIP SINGH and NIJAWAN, S. D.) . . 956

BAWEJA, KESHO DAS (AFZAL HUSAIN, M. and)—Studies on *Schistocerca gregaria* 586

PAGE

Bemisia gossypiperda, seasonal activities of, in the Punjab (AFZAL HUSAIN, M. *et al.*) 893

Bengal, a biochemical study of the paddy soils of (DE, P. K. and PAIN, A. K.) 746

BHAT, S. S. (CHEEMA, G. S. and)—Banana breeding at the Imperial College of Tropical Agriculture 484

BHATIA, D. R. (AFZAL HUSAIN, M. and)—Studies on *Schistocerca gregaria* 665

BHATNAGAR, M. P. (BOSE, R. D. and)—Studies in Indian fibre plants . 351

Bibliography of soil science, fertilizers and general agronomy (Notice) 129

Bismuthate method for the estimation of manganese in plant material (RAMJI NARAIN and AZMAT SINGH) 757

Black pattern in the Desert Locust hoppers, influence of temperature on (AFZAL HUSAIN, M. and AHMAD, TASKHIR) 624

Black pigmentation in *Schistocerca gregaria*, influence of carbon dioxide on the development of (AFZAL HUSAIN, M. and MATHUR, C. B.) . 1005

Blight and hollow-stem of sorghum (UPPAL, B. N. *et al.*) 1323

Bombay, a Rhizoctonia on sweet potatoes in (MUNDKUR, B. B.) . . 994

———, gummosis of citrus in (UPPAL, B. N. and KAMAT, M. N.) . 803

Bombay Presidency, nitrogen recuperation in the soils of (SAHASRABUDDHE, D. L. and ABHYANKAR, V. S.) 268

BORASIO, LUIGI—"Methods of Analysis and Appreciation of Wheat, Flours and Bread, particularly in respect of Investigations on the Baking Value" 866, 1134

BOSE, R. D., AZIZ, M. A. and BHATNAGAR, M. P.—"Studies in Indian Fibre Plants, No. 4. The Root-system of Sunn-hemp (*Crotalaria juncea*)" 351

BOSE, S. S. (KULKARNI, R. K. and)—Statistical notes for agricultural workers 460

——— (BANERJEE, K. C. and)—Statistical notes for agricultural workers 1122

——— (CHAKRAVERTTI, S. C. and)—Statistical notes for agricultural workers 34

———, GANGULI, P. M. and MAHALANOBIS, P. C.—"Statistical Notes for Agricultural Workers, No. 19.—The Frequency Distribution of Plot Yields and the Optimum Size of Plots in a Uniformity Trial with Rice in Assam" 1107

Burma, Cyto-genetic analysis of a type of *Saccharum spontaneum* from (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 9

C

PAGE

Cajanus indicus, wilt-resistance in (SHAW, F. J. F.) 139

Capnodium citri, causing saprophytic moulds in citrus (CHAUDHURI, H.) 97

CARBERY, M. and CHAKLADAR, M. N.—"Studies on Soil-moisture, I. Movement of Soil-moisture under Field Conditions" . . . 1201

Carbon dioxide, the influence of, on development of black pigmentation in *Schistocerca gregaria* (AFZAL HUSAIN, M. and MATHUR, C. B.) . 1005

Central Provinces, decomposition of farmyard manure and sulphate of ammonia in the paddy soils of (MUKERJI, B. K. and VISHNOI, S. L.) 17

Chaetomium sp. causing saprophytic moulds in citrus (CHAUDHURI, H.) 98

CHAKLADAR, M. N. (CARBERY, M. and)—Studies on soil-moisture . . 1201

CHAKRAVERTTI, S. C.; BOSE, S. S. and MAHALANOBIS, P. C.—"Statistical Notes for Agricultural Workers, No. 16—A Complex Experiment on Rice at the Chinsurah Farm, Bengal, 1933-34" 34

Chalcidoid parasites of *Aspidiotus orientalis*, a note on (GLOVER, P. M.) . 991

CHARLTON, J.—"Single-value Soil Properties: Moisture at the Sticky Point and *R*" 1054

CHATTERJEE, BARADANANDA (MUKHERJEE, J. N. and)—Reactions responsible for soil acidity 517

CHAUDHURI, H.—"Diseases of Citrus in the Punjab" 72

CHEEMA, G. S. and BHAT, S. S.—"Banana Breeding at the Imperial College of Tropical Agriculture, Trinidad" 484

CHINSURAH Farm, a complex experiment on rice at (CHAKRAVERTTI, S. C. *et al.*) 34

Chlorides of non-essential elements, tolerance of wheat plants for (SINGH, B. N. and PRASAD, S.) 720

Chlorosis disease of citrus (CHAUDHURI, H.). 92

Chota Nagpur, the occurrence of *Chrysomphalus aurantii* and *Laccifer lacca* on grape fruit (GLOVER, P. M.) 991

CHOWDHURY, S. C.—"A Disease of *Zea Mays* caused by *Colletotrichum graminicolum* (Ces.) Wils" 833

Chromosome studies in some Indian forms of *Saccharum spontaneum* (JANAKI AMMAL, E. K.) 1

Chrysomphalus aurantii, the occurrence of, on grape fruit in Ranchi District (GLOVER, P. M.) 991

Cicer arietinum L., inheritance of seed-characters in (HUKUM SINGH and EKBOTE, R. B.) 1087

Citrus diseases in the Punjab (CHAUDHURI, H.) 72

Citrus, gummosis of, in Bombay (UPPAL, B. N. and KAMAT, M. N.) . 803

Cladosporium herbarum var. *citricola* F. & B. causing saprophytic moulds in citrus (CHAUDHURI, H.) 98

PAGE

Classification of the autumn rices of the Punjab and Western United Provinces (KASHI RAM and EKBOTE, R. B.) 930

Clay acids, titration curves of (MITRA, RAMPRASAD) 555

Colletotrichum gloeosporioides, citrus anthracnose caused by (CHAUDHURI, H.) 75

Colletotrichum graminicolum, on *Zea Mays* (CHOWDHURY, S. C.) . . 833

Colour changes and sexual behaviour in desexualised *Schistocerca gregaria* adults (AFZAL HUSAIN, M. and BAWEJA, K. D.) 586

Cotton (Dharwar), influence of storage on the fibre properties of (NAYAK, H. R.) 52

Cotton failure in the Punjab and some abnormalities in the cotton plant (DASTUR, R. H.) 377

——— fibre, a device for separating different lengths of, from seed-cotton (MCNAMARA, H. C. and STUTTS, R. T.) 1153

——— hair, progressive damage to, caused by micro-organisms (GULATI, A. N.) 861

———, hairiness of (AFZAL, M.). 823

——— (Malvi), analysis of selection methods used in the improvement of (HUTCHINSON, J. B. and KUBERSINGH). 672

——— plant, some abnormalities in (DASTUR, R. H.) . . . 377

———, root-rot disease of, in the Punjab (VASUDEVA, R. S.) . . 904

———, seasonal activities of the White-fly of, in the Punjab (AFZAL HUSAIN, M. *et al.*) 893

———, small-leaf disease of (GOKHALE, V. P.) 475

——— soils (black), nitrogen balance in (WAD, Y. D. and AURANGABADKAR, R. K.) 316

———, variation in the fibre-properties of similar strains of, grown in different tracts of Gujarat (PATEL, B. S. and SRINAGABHUSHANA) . 63

——— (Indian), fibre-maturity in (GULATI, A. N. and AHMAD, N.) . 502

Crepis dioscoridis, inheritance of some morphological characters in (PUREWAL, S. S.) 855

Crotalaria juncea, microsporogenesis in (BANERJI, I. and SAMAL, K. K.) 116

———————, root-system of (BOSE, R. D. *et al.*) 351

Cyperus tegetiformis, a disease of, caused by *Kawakamia cyperi* (SAHAYA, C. B.) 992

Cyto-genetic analysis of *Saccharum spontaneum* (JANAKI AMMAL, E. K.) 1

——— ——————————— (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 9

D

PAGE

DALIP SINGH and NIJAWAN, S. D.—" Base-exchange Studies. I. A Preliminary Study of the Effect of certain Cations saturating the Soil's Exchange Complex on its Physico-chemical Properties, and their relation to the Plant Growth " 956

DASTUR, R. H.—" A Preliminary Note on Cotton Failure in the Punjab and some Abnormalities in the Plant ". 377

DE, PRAN KUMAR—" The Problem of the Nitrogen Supply of Rice, I. Fixation of Nitrogen in the Rice Soils under Water-logged Conditions " 1237

——————— and PAIN, ANIL KUMAR—" A Biochemical Study of the Paddy Soils of Bengal with special reference to their Nitrogen-fixing Capacities " 746

——————— " Studies on the Organic Nitrogen of Paddy Soils, Part I. Distribution of ' Humic ' and ' Non-Humic ' Nitrogen in Organic Matter " 1081

DESAI, S. V. and FAZAL-UD-DIN.—" On the Nature of Nitrification in Soil " 777

——————— " Photo-chemical Oxidation by Plant Materials " 985

Desert Locust, biology of, with special relation to temperature (AFZAL HUSAIN, M. and AHMAD, T.) 188

———————, factors determining movement of the vermiform larvae of (AFZAL HUSAIN, M. and BHATIA, D. R.) 665

———————, influence of temperature on black pattern in (AFZAL HUSAIN, M. and AHMAD, T.) 624

———————, pigmentation and physical exertion in (AFZAL HUSAIN, M. and MATHUR, C. B.) 591

———————, colour changes and sexual behaviour in desexualised adults (AFZAL HUSAIN, M. and BAWEJA, K. D.) 586

———————, influence of carbon dioxide on development of black pigmentation in (AFZAL HUSAIN, M. and MATHUR, C. B.) . . 1005

———————, the incursion of 1935 (RAMCHANDRA RAO, Y.) . . 1031

———————, why they eat wool (AFZAL HUSAIN, M. and MATHUR, C. B.) 263

DESHPANDE, R. B.—" Mendelian Segregation in *Hibiscus sabdariffa* L. in relation to the Age of the F_1 at Time of Fertilization " . . 1274

Destructive Insects and Pests Act, 1914 (corrected up to 1st October 1936) 1337

Dharwar cotton, influence of storage on the fibre properties of (NAYAK, H. R.) 52

Dimpled grains in sorghum, inheritance of (RANGASWAMI AYYANGAR, G. N. *et al.*) 938

E

PAGE

Earliness in the United Provinces rices (SETHI, R. L. *et al.*) . . . 361

———————————, inheritance of (SETHI, R. L. *et al.*) 1246

EKBOTE, R. B. (HUKUM SINGH and)—Inheritance of seed-characters in gram 1087

———————— (KASHI RAM and)—Classification of the autumn rices of the Punjab and Western United Provinces 930

Exchange complex of soils, effect of cations saturating it, on its physico-chemical properties (DALIP SINGH and NIJAWAN, S. D.) . . . 956

F

Farmyard manure, decomposition of, in the paddy soils of the Central Provinces (MUKERJI, B. K. and VISHNOI, S. L.) 17

FAZAL-UD-DIN—" Reduction of Nitrates by the Sun " 844

———————— (DESAI, S. V. and)—Photo-chemical oxidation by plant materials 985

———————————— Nitrification in soil . . . 777

Fibre-maturity of Indian cottons (GULATI, A. N. and AHMAD, N.) . . 502

————plants (Indian), studies in (BOSE, R. D. *et al.*) 351

————properties of Dharwar cotton, influence of storage on (NAYAK, H. R.) 52

———————— of similar strains of cotton grown in different tracts of Gujarat (PATEL, B. S. and SRINAGABHUSHANA) . . . 63

Fibres (cotton), a device for separating different lengths of, from seed cotton (MCNAMARA, H. C. and STUTTS, R. T.) 1153

Field experiments, factors that influence errors in, with special reference to sugarcane (SAYER, W. and KRISHNA IYER, P. V.) . . . 917

———————— with *juar*, influence of shape and size of plots on the effective precision of (KULKARNI, R. K. *et al.*) 460

Flours, wheat and bread, methods of analysis and appreciation of (LUIGI BORASIO) 866, 1134

Fungal cultures, the storage of (GALLOWAY, L. D.) 946

Fungi of Indian soils (GALLOWAY, L. D.) 578

G

GALLOWAY, L. D.—" Indian Soil Fungi " 578

———————— — " The Storage of Fungal Cultures " . . . 946

GANGULI, P. M. (BOSE, S. S. and)—Statistical notes for agricultural workers 1107

PAGE

GANGULI, S. C. (MUKHERJEE, J. N. and)—Reactions responsible for soil acidity 517

Germination of crop seeds in the field (LABH SINGH and ALAM, N.) . 784

GLOVER, P. M.—" An Account of the Occurrence of *Chrysomphalus aurantii* Mask. and *Laccifer lacca* Ker. on Grape Fruit in Ranchi District, Chota Nagpur, with a Note on the *Chalcidoid* Parasites of *Aspidiotus orientalis* Newst." (Abstract) 991

GOKHALE, V. P.—" Preliminary Observations on Small-leaf Disease in Cotton " 475

Gram, inheritance of seed characters in (HUKUM SINGH and EKBOTE, R. B.) 1087

Grape fruit, the occurrence of *Chrysomphalus aurantii* and *Laccifer lacca* on (GLOVER, P. M.) 991

Great millet (see ' sorghum ') . . . 481, 938, 1105, 1299, 1323, 1335

Gujarat, fibre-properties of similar strains of cotton grown in different tracts of (PATEL, B. S. and SRINAGABHUSHANA) 63

GULATI, A. N.—" A Note on a New Type of Progressive Damage to the Structure of Cotton Hair caused by Micro-organisms " 861

———— and AHMAD, N.—" Fibre-maturity of Indian Cottons " (Abstract) 502

Gummosis of citrus in Bombay (UPPAL, B. N. and KAMAT, M. N.) . 803

H

Hairiness of cotton (AFZAL, M.) 823

Helminthosporium spp. on sugarcane (SUBRAMANIAM, L. S.) . . . 11

Hibiscus sabdariffa, Mendelian segregation in (DESHPANDE, R. B.) . 1274

Hollow-stem and blight of sorghum (UPPAL, B. N. *et al.*) . . . 1323

Hot weather, changes in black cotton soils during (WAD, Y. D. and AURANGABADKAR, R. K.) 316

HUKUM SINGH and EKBOTE, R. B.—"The Inheritance of Seed-characters in Gram (*Cicer arietinum* L.)" 1087

Humic nitrogen, the distribution of, in organic matter of soils (DE, P. K. and PAIN, A. K.) 1081

HUTCHINSON, J. B. and KUBERSINGH.—" Studies in Plant Breeding Technique, I. An Analysis of the Efficiency of Selection Methods used in the Improvement of Malvi Cotton " 672

Hybrid of Saccharum × Sorghum (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 1105

Hybrid, sterile, between *Nicotiana Tabacum* and *N. plumbaginifolia* (PAL, B. P. and PUSHKAR NATH) 828

Hydromania of *Schistocerca gregaria*, a study in (AFZAL HUSAIN, M. and MATHUR, C. B.) 263

I

PAGE

Imperial College of Tropical Agriculture, banana breeding at the (CHEEMA, G. S. and BHAT, S. S.) 484
Indian corn (see "*Zea Mays*") 833
—— cottons, fibre-maturity of (GULATI, A. N. and AHMAD, N.) . 502
—— fibre plants, studies in (BOSE, R. D. *et al.*) 351
—— forms of *Saccharum spontaneum*, chromosome studies in (JANAKI AMMAL, E. K.) 1
—— pulses, studies in (SHAW, F. J. F.) 139
—— soil fungi (GALLOWAY, L. D.) 578
Inheritance of characters in sorghum (RANGASWAMI AYYANGAR, G. N. *et al.*) 481, 938
———— of earliness in United Provinces rices (SETHI, R. L. *et al.*) . 1246
———— of morphological characters and of wilt-resistance in *rahar* (*Cajanus indicus*) (SHAW, F. J. F.) 139
———— of seed characters in gram (HUKUM SINGH and EKBOTE, R. B.) 1087
———— of some morphological characters in *Crepis dioscoridis* (PUREWAL, S. S.) 855
Inorganic nitrogen, utilization of, during the decomposition of plant materials in the soil (SHRIKHANDE, J. G.) 767
International Botanical Congress (Sixth), resolutions carried by . . 504

J

JANAKI AMMAL, E. K.—"Cyto-genetic Analysis of *Saccharum spontaneum* L., I. Chromosome Studies in some Indian Forms" . . 1
———— and SINGH, T. S. N.—"A Preliminary Note on a New Saccharum × Sorghum Hybrid" 1105
————.—"Cyto-genetic Analysis of *Saccharum spontaneum* L., 2. A Type from Burma" . . . 9
Juar, blight and hollow-stem of (UPPAL, B. N. *et al.*) 1323
——, influence o shape and size of plots on the effective precision of field experiments with (KULKARNI R. K. *et al.*) 460
Juice of sugarcane, mineral matter in, and its effect on the recovery of white sugar (LANDER, P. E. and RAMJI NARAIN) . . . 1218

K

KAMAT, M. N. (UPPAL, B. N. and)—A new variety of *Oidiopsis taurica* 110
————————————Gummosis of citrus in Bombay . 803
KASHI RAM and EKBOTE, R. B.—"The Classification of the Autumn Rices of the Punjab and Western United Provinces" . . . 930

PAGE

KATTI, M. S. (RAMDAS, L. A. and)—Agricultural meteorology . . 1163

Kawakamia cyperi, a disease of *Cyperus tegetiformis*, caused by (SAHAYA, C. B.) 992

KHESWALLA, K. F.—" A Phoma Disease of Asparagus " . . . 800

KOLHATKAR, K. G. (UPPAL, B. N. and)—Blight and hollow-stem of sorghum 1323

KRISHNA IYER, P. V. (SAYER, W. and)—Factors that influence the error of field experiments 917

KUBERSINGH (HUTCHINSON, J. B. and)—Studies in plant breeding technique 672

KULKARNI, R. K., BOSE, SUBHENDU SEKHAR and MAHALANOBIS, P. C.—" Statistical Notes for Agricultural Workers, No. 17.—On the Influence of Shape and Size of Plots on the Effective Precision of Field Experiments with *Juar* (*Andropogon Sorghum*) " 460

KUNHIKORAN NAMBIAR, A. (RANGASWAMI AYYANGAR, G. N. and)—Inheritance of characters in sorghum 481, 938

L

LABH SINGH and ALAM, NEK.—" Studies in the Germination of Crop Seeds in the Field " 784

Laccifer lacca, occurrence on grape fruit in Ranchi District (GLOVER, P. M.) 991

LANDER, P. E. and RAMJI NARAIN.—" Mineral Matter in the Juice of Sugarcane and its Effect on the Recovery of White Sugar, I." . 1218

Locust (Desert), a study in the hydromania of (AFZAL HUSAIN, M. and MATHUR, C. B.) 263

———, factors determining movement of the vermiform larvae of (AFZAL HUSAIN, M. and BHATIA, D. R.) 665

———, incursion of 1935 in North-West India (RAMCHANDRA RAO, Y.) 1031

———, influence of temperature on black pattern in (AFZAL HUSAIN, M. and AHMAD, T.) 624

———, pigmentation and physical exertion in (AFZAL HUSAIN, M. and MATHUR, C. B.) 591

———, colour changes and sexual behaviour in desexualised adults of (AFZAL HUSAIN, M. and BAWEJA, K. D.) 580

———, influence of carbon dioxide on black pigmentation in (AFZAL HUSAIN, M. and MATHUR, C. B.) 1005

———, the biology of (AFZAL HUSAIN, M. and AHMAD, T.) . 188

Locusts, why they eat wool (AFZAL HUSAIN, M. and MATHUR, C. B.) . 263

LUTHRA, J. C. and SATTAR, A.—" Some Studies on the Sclerotial Diseases of Rice (*Sclerotium oryzae* Catt.) in the Punjab " 973

M

PAGE

MAHALANOBIS, P. C. (BANERJEE, K. C. and)—Statistical notes for agricultural workers 1122

——— (BOSE, S. S. and)—Statistical notes for agricultural workers 1107

——— (CHAKRAVERTTI, S. C. and)—Statistical notes for agricultural workers 34

——— (KULKARNI, B. K. and)—Statistical notes for agricultural workers 460

Maize, disease of, caused by *Colletotrichum graminicolum* (CHOWDHURY, S. C.) 833

Malformation of *Vitis quadrangularis*, systematic position of the smut causing (MUNDKUR, B. B.) 876

Malvi cotton, analysis of selection methods used in the improvement of (HUTCHINSON, J. B. and KUBERSINGH) 672

Malwa Plateau, nitrogen balance in black cotton soils in (WAD, Y. D. and AURANGABADKAR, R. K.) 316

Manganese in plant material, bismuthate method for the estimation of (RAMJI NARAIN and AZMAT SINGH) 757

Manure (farmyard), decomposition of, in the paddy soils of the Central Provinces (MUKERJI, B. K. and VISHNOI, S. L.) 17

MATHUR, C. B. (AFZAL HUSAIN, M. and)—Studies on *Schistocerca gregaria* 263, 591, 1005

MCNAMARA, H. C. and STUTTS—R. T.—"A Device for separating different Lengths of Fibres from Seed Cotton" (Abstract) . . 1153

MEHTA, T. R. (SETHI, R. L. and)—Earliness in the United Provinces rices 361

——— Inheritance of earliness in United Provinces rices 1246

Mendelian ratios, tables of standard errors of 878

——— segregation in *Hibiscus sabdariffa* L. in relation to the age of the F_1 at time of fertilization (DESHPANDE, R. B.) . . . 1274

Meteorological data, the use of χ^2-technique of analysis on (SUKHATME, P. V.) 1292

Meteorology, agricultural, studies in (RAMDAS, L. A. and KATTI, M. S.) . 1163

Micro-organisms, progressive damage to the structure of cotton hair caused by (GULATI, A. N.) 861

Microsporogenesis in *Crotalaria juncea* (BANERJI, I. and SAMAL, K. K.) 116

Mineral matter in the juice of sugarcane and its effect on the recovery of white sugar (LANDER, P. E. and RAMJI NARAIN) . . . 1218

MITRA, RAMPRASAD (MUKHERJEE, J. N. and)—Reactions responsible for soil acidity 517

MITRA, RAMPRASAD.—"On the Nature of Reactions responsible for Soil Acidity, Part V, Titration Curves of Clay Acids" . . 555

PAGE

Moisture in soils at sticky point and *R* (CHARLTON, J.). 1054

Moisture in the surface layers of the atmosphere, soil moisture in relation to (RAMDAS, L. A. and KATTI, M. S.) 1163

Morphological characters, inheritance of, in *rahar* (*Cajanus indicus*) (SHAW, F. J. F.) 139

MUKERJI, B. K. and VISHNOI, S. L.—"A Bio-chemical Study of the Decomposition of Farmyard Manure and Sulphate of Ammonia in the Paddy Soils of the Central Provinces" 17

MUKHERJEE, JNANENDRANATH; MITRA, RAMPRASAD; GANGULI, SANTOSH CHANDRA and CHATTERJEE, BARADANANDA.—"On the Nature of Reactions responsible for Soil Acidity, Part IV. Interpretation of Titration Curves" 517

MUNDKUR, B. B.—"A Rhizoctonia on Sweet Potatoes in Bombay" . 994

———— "On the Systematic Position of the Smut causing Malformation of *Vitis quadrangularis*". 876

Mutant, brownish purple, in sorghum (RANGASWAMI AYYANGAR, G. N. and KUNHIKORAN NAMBIAR, A.) 481

Mycosyrinx arabica (Henn.), smut causing malformation of *Vitis quadrangularis* (MUNDKUR, B. B.) 876

N

NAYAK, H. R.—"Influence of Storage on some of the Fibre-properties of Dharwar Cotton" 52

Nicotiana plumbaginifolia and *N. Tabacum*, a sterile hybrid between (PAL, B. P. and PUSHKAR NATH) 828

Nicotiana Tabacum and *N. plumbaginifolia*, a sterile hybrid between (PAL, B. P. and PUSHKAR NATH) 828

NIJAWAN, S. D. (DALIP SINGH and)—Base-exchange studies . . 956

Nitrates, reduction of, by the sun (FAZAL-UD-DIN) 844

Nitrification in soil (DESAI, S. V. and FAZAL-UD-DIN). 777

Nitrogen balance in black cotton soils in the Malwa Plateau (WAD, Y. D. and AURANGABADKAR, R. K.) 316

Nitrogen-fixing capacities of the paddy soils of Bengal (DE, P. K. and PAIN, A. K.) 746

Nitrogen (humic and non-humic), distribution of, in organic matter of soils (DE, P. K. and PAIN, A. K.) 1081

———— (inorganic), utilization of, during the decomposition of plant materials in the soil (SHRIKHANDE, J. G.) 767

———— in rice soils, fixation of, under water-logged conditions (DE, P. K.) 1237

———— (organic) of paddy soils (DE, P. K. and PAIN, A. K.) . . 1081

———— recuperation in the soils of the Bombay Presidency (SAHASRABUDDHE, D. L. and ABHYANKAR, V. S.) 268

PAGE

Nitrogen supply of rice, the problem of (DE, P. K.) 1237
Non-humic nitrogen, the distribution of, in organic matter of soils (DE, P. K. and PAIN, A. K.) 1081
North-West India, the locust incursion of 1935 in (RAMCHANDRA RAO, Y.) 1031

O

Oidiopsis taurica, a new variety of (UPPAL, B. N. *et al.*) . . . 110
Organic matter of soils, distribution of humic and non-humic nitrogen in (DE, P. K. and PAIN, A. K.) 1081
——— nitrogen of paddy soils (DE, P. K. and PAIN, A. K.) . . 1081
Oryza sativa (see "Rice") . 17, 34, 361, 396, 746, 930, 973, 1081, 1107, 1237, 1246

P

Paddy (see "Rice") . 17, 34, 361, 396, 746, 930, 973, 1081, 1107, 1237, 1246
PAIN, A. K. (DE, P. K. and)—Paddy soils of Bengal. . . . 746
——————Studies on the organic nitrogen of paddy soils 1081
PAL, B. P.—"Effects of Brown Rust Attack on Wheat" . . . 127
——— and PUSHKAR NATH.—"A Note on the Sterile Hybrid between *Nicotiana Tabacum* L. and *N. plumbaginifolia* Viv." . . 828
Panama disease of banana, breeding with a view to obtain a type resistant to (CHEEMA, G. S. and BHAT, S. S.) 484
PANDURANGA RAO, V. (RANGASWAMI AYYANGAR, G. N. and)—Inheritance of characters in sorghum 938
——————— Studies in sorghum 1299
PATEL, B. S. and SRINAGABHUSHANA.—"Variation in the Fibre-properties of similar Strains of Cotton grown in different Tracts of Gujarat" 63
PATEL, M. K. (UPPAL, B. N. and)—A new variety of *Oidiopsis taurica* 110
——————— Blight and hollow-stem of sorghum 1323
Phoma disease of asparagus (KHESWALLA, K. F.) 800
Photo-chemical oxidation by plant materials (DESAI, S. V. and FAZAL-UD-DIN) 985
Physical exertion and pigmentation in *Schistocerca gregaria* (AFZAL HUSAIN, M. and MATHUR, C. B.) 591
Pigeon-pea (*Cajanus indicus*), wilt resistance in (SHAW, F. J. F.) . . 139
Pigmentation and physical exertion in *Schistocerca gregaria* (AFZAL HUSAIN, M. and MATHUR, C. B.) 591

PAGE

Pigmentation (black) in *Schistocerca gregaria*, influence of carbon dioxide on the development of (AFZAL HUSAIN, M. and MATHUR, C. B.) 1005

Plant breeding technique, studies in (HUTCHINSON, J. B. and KUBER-SINGH) 672

——— growth, relation of cations saturating the soil's exchange complex to (DALIP SINGH and NIJAWAN, S. D.) 956

——— material, bismuthate method for the estimation of manganese in (RAMJI NARAIN and AZMAT SINGH) 757

——— materials in the soil, utilization of inorganic nitrogen during the decomposition of (SHRIKHANDE, J. G.) 767

———————, photo-chemical oxidation by (DESAI, S. V. and FAZAL-UD-DIN) 985

Pleospora herbarum, causing saprophytic moulds in citrus (CHAUDHURI, H.) 98

Plot yields and the optimum size of plots in a uniformity trial with rice in Assam (BOSE, S. S. *et al.*) 1107

Pollen in sorghum (RANGASWAMI AYYANGAR, G. N. and PANDURANGA RAO, V.) 1299

PRASAD, S. (SINGH, B. N. and)—Tolerance of wheat plants for chlorides of non-essential elements 720

Pulses (Indian), studies in (SHAW, F. J. F.) 139

Punjab, classification of the autumn rices of (KASHI RAM and EKBOTE, R. B.) 930

———, cotton failure in, and some abnormalities in the plant (DASTUR, R. H.) 377

———, diseases of citrus in the (CHAUDHURI, H.) 72

———, root-rot disease of cotton in (VASUDEVA, R. S.) 904

———, seasonal activities of White-fly of cotton in (AFZAL HUSAIN, M. *et al.*) 893

———, sclerotial diseases of rice in (LUTHRA, J. C. and SATTAR, A.) . 973

PUREWAL, SWARN SINGH.—"Inheritance of some Morphological Characters in *Crepis dioscoridis*" 855

Pusa, tonnage experiments with Co 205 and Co 213, in (SAYER, W. *et al.*) 684

PUSHKAR NATH (PAL, B. P. and)—Sterile hybrid between *Nicotiana Tabacum* and *N. plumbaginifolia* 828

Pythium sp. on sugarcane (SUBRAMANIAM, L. S.) 13

PAGE

Rahar (*Cajanus indicus*), wilt-resistance in (SHAW, F. J. F.) . . 139

RAMCHANDRA RAO, Y.—"The Locust Incursion of 1935 in North-West India—Its Significance in the Study of the Locust Problem" . 1031

RAMDAS, L. A. and KATTI, M. S.—"Agricultural Meteorology: Studies on Soil-moisture in relation to Moisture in the Surface Layers of the Atmosphere during the Clear Season at Poona" 1163

RAMJI NARAIN and AZMAT SINGH.—"Bismuthate Method for the Estimation of Manganese in Plant Material" 757

——— (LANDER, P. E. and).—Mineral matter in the juice of sugarcane 1218

Ranchi District, the occurrence of *Chrysomphalus aurantii* and *Laccifer lacca* on grape fruit in (GLOVER, P. M.) 991

RANGASWAMI AYYANGAR, G. N. and KUNHIKORAN NAMBIAR, A.—"Inheritance of Characters in Sorghum—the Great Millet, VIII. A Brownish Purple Mutant" 481

——— and PANDURANGA RAO, V.—"Studies in Sorghum—the Great Millet, III. Anther, Pollen and Stigma" . 1299

———; SANKARA AYYAR, M. A.; PANDURANGA RAO, V. and KUNHIKORAN NAMBIAR, A.—"Inheritance of Characters in Sorghum—the Great Millet, IX. Dimpled Grain" . 938

Recuperation of nitrogen in the soils of the Bombay Presidency (SAHASRABUDDHE, D. L. and ABHYANKAR, V. S.) . . . 268

Reduction of nitrates by the sun (FAZAL-UD-DIN) 844

REVIEWS—

Vernalization and Phasic Development of Plants 882

A Comparative Study of certain Morphological Characters of Sugarcane × Sorgo Hybrids 996

Plant Breeding Abstracts Supplement II: Summary of Reports received from Stations in the British Empire, 1932-35 . . 1155

The Cultivated Races of Sorghum 1335

Flour Milling Processes 1335

Rhizoctonia bataticola, causing root-rot disease of cotton in the Punjab (VASUDEVA, R. S.) 904

Rhizoctonia on sweet potatoes in Bombay (MUNDKUR, B. B.) . . 994

Rhizoctonia solani, causing root-rot of cotton in the Punjab (VASUDEVA, R. S.) 904

Rice, a complex experiment on (CHAKRAVERTTI, S. C. *et al.*) . . 34

—— grain, developmental variation in (TIN, U) 396

——, sclerotial disease of, in the Punjab (LUTHRA, J. C. and SATTAR, A.) 973

——, the frequency distribution of plot yields and optimum plot size in a uniformity trial with (BOSE, S. S. *et al.*) 1107

PAGE

Rice, the problem of the nitrogen supply of (DE, P. K.) . . . 1237
—— soils, decomposition of farmyard manure and sulphate of ammonia in (MUKERJI, B. K. and VISHNOI, S. L.) 17
————, fixation of nitrogen in, under water-logged conditions (DE, P. K.) 1237
———— of Bengal, bio-chemical study of (DE, P. K. and PAIN, A. K.) . 746
————, organic nitrogen of (DE, P. K. and PAIN, A. K.) . . 1081
Rices (autumn) of the Punjab and Western United Provinces, the classification of (KASHI RAM and EKBOTE, R. B.) 930
—— of United Provinces, earliness in (SETHI, R. L. *et al.*) . . 361, 1246
Root-rot disease of cotton in the Punjab (VASUDEVA, R. S.) . . 904
Root-system of sunn-hemp (BOSE, R. D. *et al.*) 351
Rust (brown) attack on wheat (PAL, B. P.) 127

S

Saccharum spontaneum, cyto-genetic analysis of (JANAKI AMMAL, E. K.) . 1
———————————— (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 9
Saccharum × sorghum hybrids (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 1105
SAHASRABUDDHE, D. L. and ABHYANKAR, V. S.—"Nitrogen Recuperation in the Soils of the Bombay Presidency, Part IV" . . . 268
SAHAYA, C. B.—"A Disease of *Cyperus tegetiformis* Roxb. caused by *Kawakamia cyperi* (Miyabe and Ideta) Miyabe" 992
SAMAL, K. K. (BANERJI, I. and)—Microsporogenesis in *Crotalaria juncea* 116
SANKARA AYYAR, M. A. (RANGASWAMI AYYANGAR, G. N. and)—Inheritance of characters in sorghum 938
Sann-hemp (*Crotalaria juncea*), microsporogenesis in (BANERJI, I. and SAMAL, K. K.) 116
————————————, root-system of (BOSE, R. D. *et al.*). . 351
Saprophytic moulds of citrus (CHAUDHURI, H.) 95
SATTAR, A. (LUTHRA, J. C. and)—Sclerotial diseases of rice in the Punjab 973
SAYER, W. and KRISHNA IYER, P. V.—"On Some of the Factors that Influence the Error of Field Experiments with special reference to Sugarcane" 917
———— ; VAIDYANATHAN, M. and SUBRAMONIA IYER, S.—"Ideal Size and Shape of Sugarcane Experimental Plots based upon Tonnage Experiments with Co 205 and Co 213 conducted in Pusa" . 684
Schistocerca gregaria, a study in the hydromania of (AFZAL HUSAIN, M. and MATHUR, C. B.) 263
————————, colour changes and sexual behaviour in desexualised adults of (AFZAL HUSAIN, M. and BAWEJA, K. D.) . . 586

PAGE

Schistocerca gregaria, why they eat wool (AFZAL HUSAIN, M. and MATHUR, C. B.) 263

————————, factors determining the movement of vermiform larvae of (AFZAL HUSAIN, M. and BHATIA, D. R.) 665

————————, influence of temperature on black pattern in (AFZAL HUSAIN, M. and AHMAD, T.) 624

———————— (locust), incursion of, in North-West India (RAMCHANDRA RAO, Y.) 1031

————————, pigmentation and physical exertion in (AFZAL HUSAIN, M. and MATHUR, C. B.) 591

————————, Influence of carbon dioxide on black pigmentation in (AFZAL HUSAIN, M. and MATHUR, C. B.) 1005

————————, the biology of (AFZAL HUSAIN, M. and AHMAD T.) 188

Sclerospora indica, merging of, with *Scl. philippinensis* (UPPAL, B. N. and WESTON, WM. H.) 715

Sclerospora philippinensis, basis for merging *Scl. indica* with (UPPAL, B. N. and WESTON, WM. H.) 715

Sclerotial diseases of rice in the Punjab (LUTHRA, J. C. and SATTAR, A.) 973

Sclerotium oryzae on rice in the Punjab (LUTHRA, J. C. and SATTAR, A.) 973

Seed-characters in gram, the inheritance of (HUKUM SINGH and EKBOTE, R. B.) 1087

Seedling diseases of sugarcane (SUBRAMANIAM, L. S.) 11

Seeds of crops, germination of, in the field (LABH SINGH and ALAM, N.) 784

SEN, A.—" Rapid Determination of Soil-moisture by Drying the Soil over Bunsen Burner " 1076

SETHI, B. L. (SETHI, R. L. and)—Earliness in the United Provinces rices 361

———————— Inheritance of Earliness in United Provinces rices 1246

SETHI, R. L.; SETHI, B. L. and MEHTA, T. R.—" Earliness in the United Provinces Rices " 361

———————— " Inheritance of Earliness in United Provinces Rices, I " 1246

Sexual behaviour and colour changes in desexualised *Schistocerca gregaria* adults (AFZAL HUSAIN, M. and BAWEJA, K. D.) . . 586

Shape and size of plots, influence of, on the effective precision of field experiments with *juar* (KULKARNI, R. K. *et al.*) 460

SHAW, F. J. F.—" Studies in Indian Pulses: the Inheritance of Morphological Characters and of Wilt Resistance in *Rahar* (*Cajanus indicus* Spreng.) " 139

PAGE

SHRIKHANDE, JAGESHWAR GOPAL.—"Utilization of certain Forms of Inorganic Nitrogen during the Decomposition of Plant Materials in the soil" 767

SINGH, B. N. and PRASAD, S.—"The Tolerance of Wheat Plant for Chlorides of certain Non-essential Elements" 720

SINGH, T. S. N. (JANAKI AMMAL, E. K. and).—A new saccharum × sorghum hybrid 1105

———————————————Cyto-genetic analysis of *Saccharum spontaneum* 9

Single-value soil properties (CHARLTON, J.) 1054

Size and shape of sugarcane experimental plots based on tonnage experiments with Co 205 and Co 213 (SAYER, W. *et al.*) 684

Small-leaf disease of cotton (GOKHALE, V. P.) 475

Smut causing malformation of *Vitis quadrangularis*, systematic position of (MUNDKUR, B. B.) 876

Soil acidity, reactions responsible for (MITRA, RAMPRASAD) 555

——————————————— (MUKHERJEE, J. N. *et al.*) . . 517

Soil, exchange complex in, effect of cations saturating it, on its physico-chemical properties, and their relation to the plant growth (DALIP SINGH and NIJAWAN, S. D.) 956

—— fungi (Indian) (GALLOWAY, L. D.) 578

—— moisture, in relation to moisture in the surface layers of the atmosphere during the clear season at Poona (RAMDAS, L. A. and KATTI, M. S.) 1163

————————, movement of, under field conditions (CARBERY, M. and CHAKLADAR, M. N.) 1201

————————, rapid determination of, by drying the soil over Bunsen burner (SEN, A.) 1076

——, nitrification in (DESAI, S. V. and FAZAL-UD-DIN). 777

——, rapid determination of moisture in (SEN, A.) 1076

——, single-value properties of (CHARLTON, J.) 1054

——, utilization of inorganic nitrogen during the decomposition of plant materials in the (SHRIKHANDE, J. G.) 767

—— (cotton, black), nitrogen balance in (WAD, Y. D. and AURANGABADKAR, R. K.) 316

Soils of the Bombay Presidency, nitrogen recuperation in (SAHASRABUDDHE, D. L. and ABHYANKAR, V. S.) 268

—— (paddy), decomposition of farmyard manure and sulphate of ammonia in (MUKERJI, B. K. and VISHNOI, S. L.) 17

————————, of Bengal, biochemical study of (DE, P. K. and PAIN, A. K.) 746

————————, organic nitrogen of (DE, P. K. and PAIN, A. K.) . . 1081

—— (rice), fixation of nitrogen in, under water-logged conditions (DE, P. K.) 1237

PAGE

Sorghum, anther, pollen and stigma in (RANGASWAMI AYYANGAR, G. N. and PANDURANGA RAO, V.) 1299
————, blight and hollow-stem of (UPPAL, B. N. *et al.*) . . . 1323
————, inheritance of characters in, (RANGASWAMI AYYANGAR, G. N. *et al.*) 481, 938
———— × saccharum hybrid (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 1105
South Africa Government, proclamation for the protection of the sugarcane crops 878
SRINAGABHUSHANA (PATEL, B. S. and)—Fibre-properties of similar strains of cotton in different tracts 63
Standard errors of Mendelian ratios, tables of 878
Statistical notes for agricultural workers (BANERJEE, K. C. *et al.*) . 1122
———— (BOSE, S. S. *et al.*). . . 1107
———— (CHAKRAVERTTI, S. C. *et al.*) . 34
———— (KULKARNI, R. K. *et al.*) . 460
Sticky point, moisture in soils at, and '*R*' (CHARLTON, J.) . . . 1054
Stigma in sorghum (RANGASWAMI AYYANGAR, G. N. and PANDURANGA RAO, V.) 1299
Storage of Dharwar cotton, influence on fibre-properties (NAYAK, H. R.) 52
Storage of fungal cultures (GALLOWAY, L. D.) 946
STUTTS, R. T. (MCNAMARA, H. C. and)—A device for separating different lengths of fibres from seed cotton 1153
SUBRAMANIAM, L. S.—"Some New Seedling Diseases of Sugarcane" . 11
SUBRAMONIA IYYAR, S. (SAYER, W. and)—Ideal size and shape of sugarcane experimental plots 684
Sugarcane experimental plots, ideal size and shape of (SAYER, W. *et al.*) 684
————, factors that influence the error of field experiments with (SAYER, W. and KRISHNA IYER, P. V.) 917
———— juice, mineral matter in, and its effect on the recovery of white sugar (LANDER, P. E. and RAMJI NARAIN) . . . 1218
————, some new seedling diseases of (SUBRAMANIAM, L. S.) . . 11
———— (saccharum) × sorghum hybrid (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 1105
———— (*Saccharum spontaneum*), cyto-genetic analysis of (JANAKI AMMAL, E. K.) . . . 1
———— (JANAKI AMMAL, E. K. and SINGH, T. S. N.) 9

PAGE

Sugar (white), effect of mineral matter on the recovery of (LANDER, P.E. and RAMJI NARAIN) 1218

SUKHATME, P. V.—"An Illustration of the Use of the χ^2-Technique of Analysis on Meteorological Data" 1292

Sulphate of ammonia, decomposition of, in the paddy-soils (MUKERJI, B. K. and VISHNOI, S. L.) 17

Sun, reduction of nitrates by the (FAZAL-UD-DIN) 844

Sweet potatoes, Rhizoctonia on, in Bombay (MUNDKUR, B. B.) . . 994

T

Temperature, influence of, on black pattern in the Desert Locust (AFZAL HUSAIN, M. and AHMAD, T.) 624

Tiller variation, studies in (BANERJEE, K. C. *et al.*) 1122

TIN, U (1).—"Developmental Variation in the Paddy Grain" . . 396

Titration curves of clay acids (MITRA, RAMPRASAD) 555

————, interpretation of (MUKHERJEE, J. N. *et al.*) . . 517

Tobacco (see '*Nicotiana Tabacum*') 828

Tolerance of wheat plants for chlorides of non-essential elements (SINGH, B. N. and PRASAD, S.) 720

Tonnage experiments with Co 205 and Co 213, ideal size and shape of experimental plots for (SAYER, W. *et al.*) 684

TREHAN, K. N. (AFZAL HUSAIN, M. and)—Studies on *Bemisia gossypiperda* 893

Trinidad, Imperial College of Tropical Agriculture, banana breeding at (CHEEMA, G. S. and BHAT, S. S.) 484

U

United Provinces rices, earliness in (SETHI, R. L. *et al.*) . . . 361

————, inheritance of earliness in (SETHI, R. L. *et al.*). 1246

———— (Western), classification of the autumn rices of (KASHI RAM and EKBOTE, R. B.) 930

UPPAL, B. N. and KAMAT, M. N.—"Gummosis of Citrus in Bombay" 803

———— and PATEL, M. K.—"A New Variety of *Oidiopsis taurica*" 110

———— and WESTON, WM. H. (JR.).—"The Basis for Merging *Sclerospora indica* with *Scl. philippinensis*" 715

————; KOLHATKAR, K. G. and PATEL, M. K.—"Blight and Hollow-stem of Sorghum" 1323

V

VAIDYANATHAN, M. (SAYER, W. and)—Ideal size and shape of sugarcane experimental plots 684

Variation (developmental), in paddy grain (TIN, U) 396

PAGE

Variation (tiller), studies in (BANERJEE, K. C. *et al.*) 1122

VASUDEVA, R. S.—"Studies on the Root-rot Disease of Cotton in the Punjab, II. Some Studies in the Physiology of the Causal Fungi" . 904

VERMA, P. M. (AFZAL HUSAIN, M. and)—Studies on *Bemisia gossypiperda* 893

Vermiform larvae of *Schistocerca gregaria*, factors determining the movement of (AFZAL HUSAIN, M. and BHATIA, D. R.) . . 665

VISHNOI, S. L. (MUKERJI, B. K. and)—Decomposition of farmyard manure and sulphate of ammonia in the paddy soils of the Central Provinces 17

Vitis quadrangularis, systematic position of the smut causing malformation of (MUNDKUR, B. B.) 876

W

WAD, YESHWANT D. and AURANGABADKAR, R. K.—"Nitrogen Balance in Black Cotton Soils in the Malwa Plateau, II. Changes during the Hot Weather" 316

Water-logged rice soils, fixation of nitrogen in (DE, P. K.) . . . 1237

WESTON, WM. H. (JR.) (UPPAL, B. N. and)—Basis for merging *Sclerospora indica* with *Scl. philippinensis* 715

Wheat, brown rust attack on (PAL, B. P.) 127

———, flours and bread, methods of analysis and appreciation of (BORASIO, LUIGI) 866, 1134

Wheat plants, tolerance of, for chlorides of non-essential elements (SINGH, B. N. and PRASAD, S.) 720

White-fly of cotton, seasonal activities of, in the Punjab (AFZAL HUSAIN, M. *et al.*) 893

Wilt-resistance in *rahar* (*Cajanus indicus*), inheritance of (SHAW, F. J. F.) 139

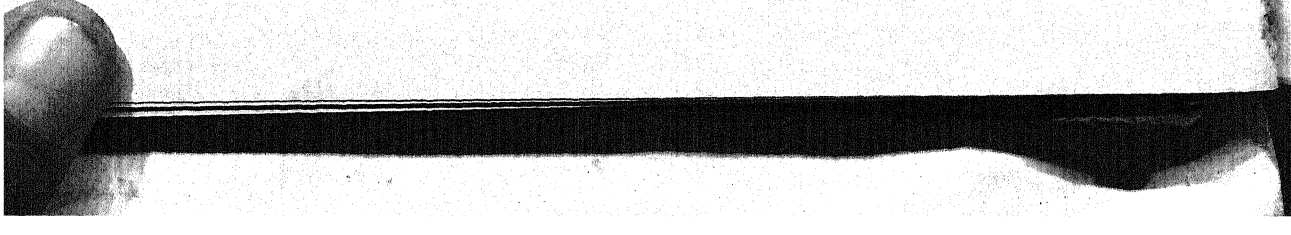
Wither-tip disease of citrus (CHAUDHURI, H.) 75

Wool, why locusts eat it (AFZAL HUSAIN, M. and MATHUR, C. B.) . 263

Z

Zea Mays, a disease of, caused by *Colletotrichum graminicolum* (CHOWDHURY, S. C.) 833

GIPD—S 3—560 IC of AR—17-2-38—800.



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ORIGINAL ARTICLES

CYTO-GENETIC ANALYSIS OF *SACCHARUM SPONTANEUM* L.

1.—CHROMOSOME STUDIES IN SOME INDIAN FORMS

BY

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(Received for publication on 15th August 1935)

(With Plates I-V)

CONTENTS

		Page
I.	Introduction	1
II.	Material and methods	2
III.	The chromosome complement in *Saccharum spontaneum*	3
IV.	Pollen mother-cell division	3
V.	Evidence of hybridity within the species	5
VI.	Conclusions	7
VII.	Summary	8
	References	8

I. Introduction

The use of *Saccharum spontaneum* L. in sugarcane breeding has been fully justified by the results obtained both in India and Java. Beginning with Co. 205, which is an inter-specific hybrid between the thick cane "Vellai" and the local form of *S. spontaneum*, most of the canes produced at Coimbatore have in them traces of *spontaneum* 'blood'.

S. spontaneum is widely distributed in the Old World. It extends from Africa through Southern Asia to Australia and is represented in India by a number of distinct forms. Hackel classified the Indian varieties into,

Sub-species (*a*) *Indicum* with very narrow lamina and slender racemes ;
Sub-species (*b*) *Oegyptiacum* with broader lamina and dense racemes.

He mentions the prevalence of " intermediates " between these two sub-species. Hole [1911], while recognizing these different forms, considers them as purely ecological types.

The method of approaching taxonomic problems has changed considerably within the last decade and additional evidence of cytology and genetics is now brought to bear on the purely morphological studies on which species were hitherto differentiated. Our concept of species has, therefore, been very much widened.

In the present study an attempt is made to bring together observations on the chromosome behaviour of some of the Indian forms of *S. spontaneum* in the hope that the cytological facts presented will help to throw light on the phylogeny of this widely-distributed Linnean species.

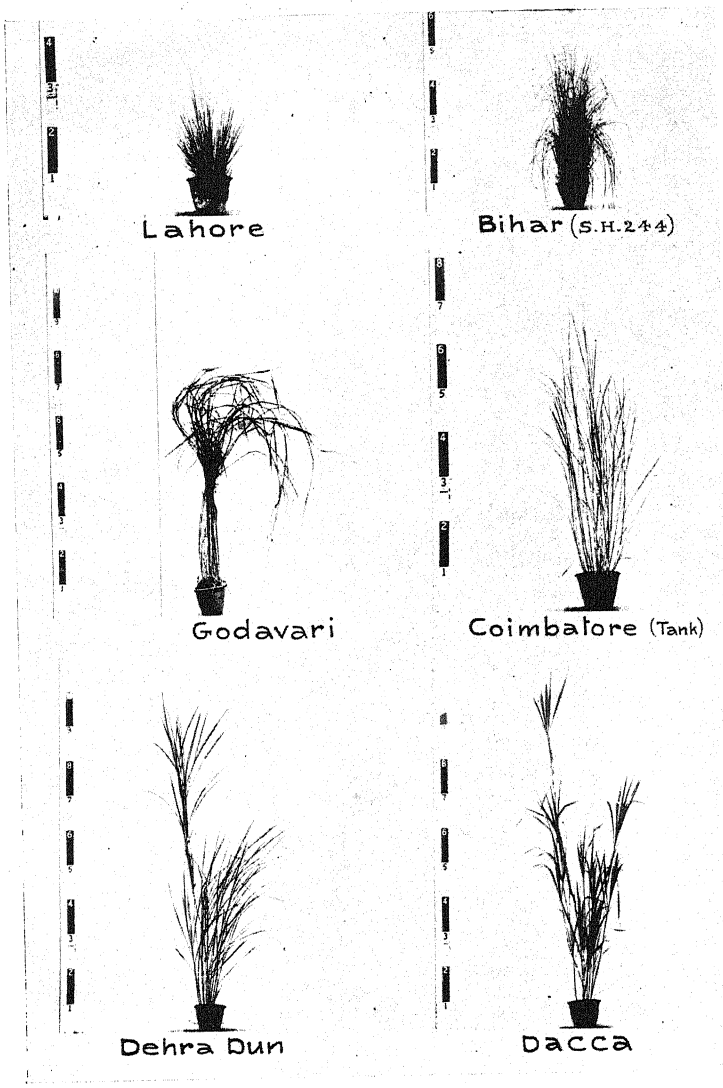
II. Material and methods

The material studied was grown at the Imperial Sugarcane Breeding Station, Coimbatore. The different types are named after the places from which they were collected. The following types were examined :—

Lahore,
Dehra Dun.
Coimbatore (Tank).
Godavari (Rellagaddi).
Dacca.
Bihar (S. H. 244 and S. H. 248).
Cochin.

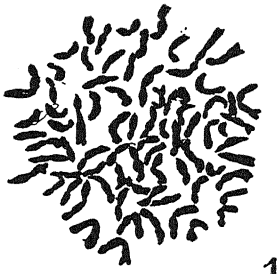
Morphological descriptions of the first five forms are included in Panje's " Studies in *Saccharum spontaneum* " [1933]. Except in the case of Lahore, which does not arrow at Coimbatore, both root tips and pollen mother-cells were studied. Root tips were fixed in Allen's modification of Bouin's fixative, PFA 15b [McClung, 1929] and in La Cour's 2 BD [La Cour, 1931]. Of these two fixatives, Bouin's was found more satisfactory for the study of metaphase plates. Freezing the root tips for about two to three minutes just before fixing, by surrounding them with crushed ice, had the effect of spacing the chromosomes evenly, and facilitated their counting. This is very important in a genus like *Saccharum* where the chromosomes are many.

Spikelets at the right stage were selected after preliminary examination of anthers in aceto-carmine [Belling, 1923]. They were then fixed in Allen's modification of Bouin's fixative (PFA 15b), and acetic alcohol in the proportion of one part acetic acid to three parts absolute alcohol. Materials fixed in acetic alcohol and preserved in 70 per cent alcohol proved excellent for aceto-carmine smears, even after a period of several months. An exhaust pump was used to hasten penetration.



Some Indian types of *Saccharum spontaneum*.

SOMATIC METAPHASE IN SOME INDIAN TYPES OF *SACCHARUM SPONTANEUM* L.



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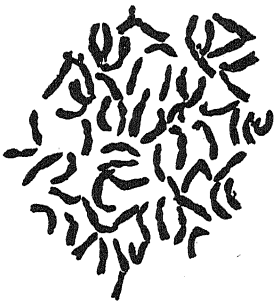

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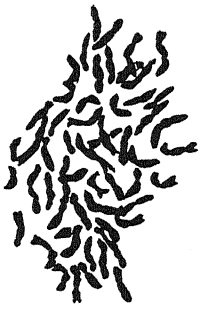

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FIG. 1. Dacca $2n=80$.

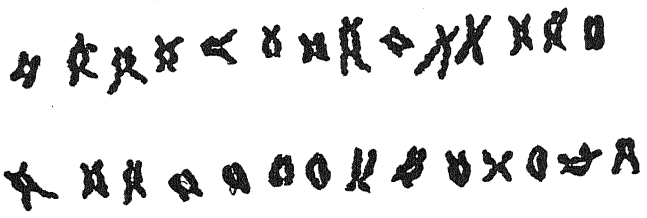
FIG. 2. Bihar $2n=64$.

FIG. 3. Rellagaddi $2n=64$.

FIG. 4. Dehra Dun $2n=56$.

FIG. 5. Lahore $2n=48$.

CHROMOSOME ASSOCIATION IN THE DEHRA DUN AND GODAVARI FORMS



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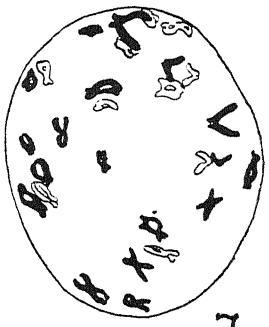

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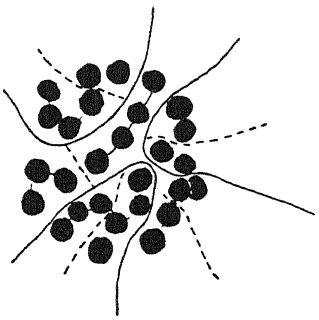

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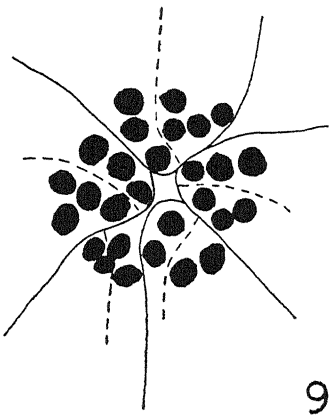

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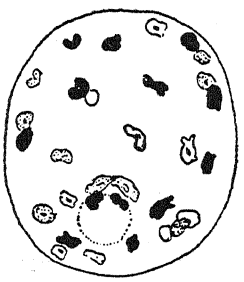

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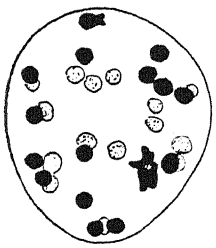

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FIG. 6. Bivalents from one P.M.C. of the Dehra Dun form $n=28$ (aceto-carmine smear).

FIG. 7. Diakinesis in the Dehra Dun form.

FIGS. 8 & 9. Prometaphase & metaphase stages in the Dehra Dun form showing "Secondary association."

FIG. 10. Early anaphase in Rellagaddi ($n=32$).

FIG. 11. Diakinesis in Rellagaddi.

FIG. 12. Prometaphase in Rellagaddi.

Sections were cut, 10μ in the case of root tips and 12 to 15μ in the case of anthers. Iron alum haematoxylin with picric acid as a de-stainer gave clearer differentiation than Iodine gentian violet.

Drawings were made at bench level with a Spencer Abbe camera lucida, a Leitz objective, 1·92 (N.A. 1·32) and a x25 eye piece, to give a magnification of 4000. For the study of aceto-carmine smears a water immersion objective (N. A. 1·2) was used.

III. The chromosome complement in *Saccharum spontaneum*

Cytological studies in *S. spontaneum* were first made by Bremer [1925] who gives the chromosome number of the Java form (Glagah) $n = 56$ and of the Celebes form " Glagah Tabongo " $n = 40$.

Chromosome counts of some of the types examined have already been published. Dutt and Rao [1932] were the first to point out that the Coimbatore form of *S. spontaneum* differs from the Java and the Celebes forms in having sixty-four chromosomes in its somatic cells. Singh [1934] reported thirty-two bivalents in the Godavari form, twenty-seven bivalents in the Dehra Dun and thirty-nine in Dacca. I find the following numbers in the types examined by me :—

	Somatic chromosomes
Lahore	48
Dehra Dun (Holes I)	56
Coimbatore (Tank)	64
Rellagaddi	64
Bihar (S. H. 244)	64
Bihar (S. H. 248)	64
Cochin	64
Dacca	80
A seedling from the Dacca form	80

The above observations suggest that the different types of *Saccharum spontaneum* represent a polyploid series with a basic number eight.

Plate II, figs. 1 to 5 give the somatic metaphase plates of the ' chromosal ' types studied. The chromosomes are found to vary both in size and morphology—at least three types can be distinguished and possibly five in some of the varieties. Both medium and secondary constrictions are found. There is indication of the occurrence of trabants in a few chromosomes of some of the varieties examined.

IV. Pollen mother-cell division

The general facts regarding meiosis are the same for all the types studied and to avoid repetition the cytology of the Dehra Dun form is given in some detail, as the one having the smallest number of chromosomes next to Lahore. This

form from Lahore—which has little or no lamina—has not so far produced any arrows in Coimbatore. It was, therefore, not possible to include in this study, observations on its meiosis.

Early prophase studies have not been attempted. Observations herein recorded have been mainly on the stages from early diakinesis or diplotene to metaphase.

(*a*) *The Dehra Dun form*

At zygotene, single threads are seen lying side by side in various grades of proximity and at diplotene loops are formed with several points of contact which are found to be ' chiasmata ' or exchange of partners between the four chromatids. An examination of early diakinesis shows the fifty-six chromosomes associated into twenty-eight bivalents by means of chiasmata (Plate III, figs. 6 and 7).

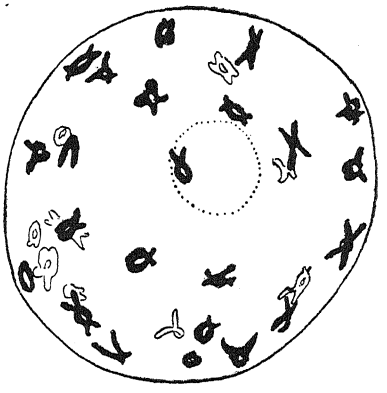
Bivalents are of two kinds, some with one chiasmata and others with two. In the case of bivalents with single chiasmata, the chiasma are localized and medium. Terminalization seldom occurs in these. They have the appearance of X's even in late diakinesis. In those with two chiasmata, terminalization is complete in the smaller chromosomes, which have the appearance of O's. Chiasmata observed at late diakinesis are never completely terminalized. Contraction of chromosomes is very intense in diakinesis. This may have been intensified by the reagent used.

With the termination of diakinetic repulsion there is a sudden convergence of the bivalents to the centre of the nucleus. From a study of large numbers of pollen mother-cells at this (pro-metaphase) stage the existence of "secondary association" of bivalents is very clear. The bivalents arrange themselves into ten groups (Plate III, fig. 8). Due partly to their close association and partly as an artefact of fixation there is generally a thin strand of linen connecting the bivalents of a group. These bivalents orient themselves to form the characteristic metaphase plate (Plate III, fig. 9). In a polar view this "secondary association" can be clearly observed. The chromosomes are very much condensed at this stage and look like spherical bodies. Inter-kinesis is short and at the second metaphase, the chromosomes are found to be still associated. Tetrad formation is normal.

(*b*) *The Godavari form* (*Rellagaddi*) 2n=*64*

This is the form of *S. spontaneum* growing on the banks of the Godavari river. Its chromosome number reported by Singh [1934] is $n=32$, which I have also observed (Plate III, fig. 10). Plate III, fig. 11 shows a pollen mother-cell at late diakinesis. Thirty-two bivalents are generally observed, but in a number of nuclei examined at this stage, a single tetravalent could be observed. The number of bivalents with single chiasmata are slightly less than those in the Dehra Dun form. As pairing akes place not between chromosomes but blocks of chromosomes

CHROMOSOME ASSOCIATION IN THE BIHAR AND DACCA FORMS



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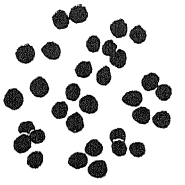

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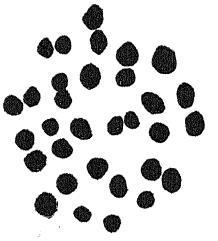

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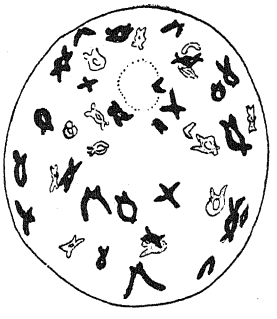

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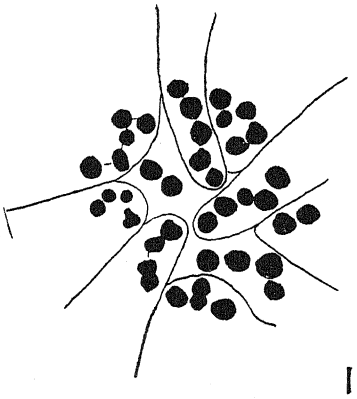

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FIG. 13. P.M.C. at diakinesis stage in the Bihar form showing 28 bivalents (aceto-carmine smear).

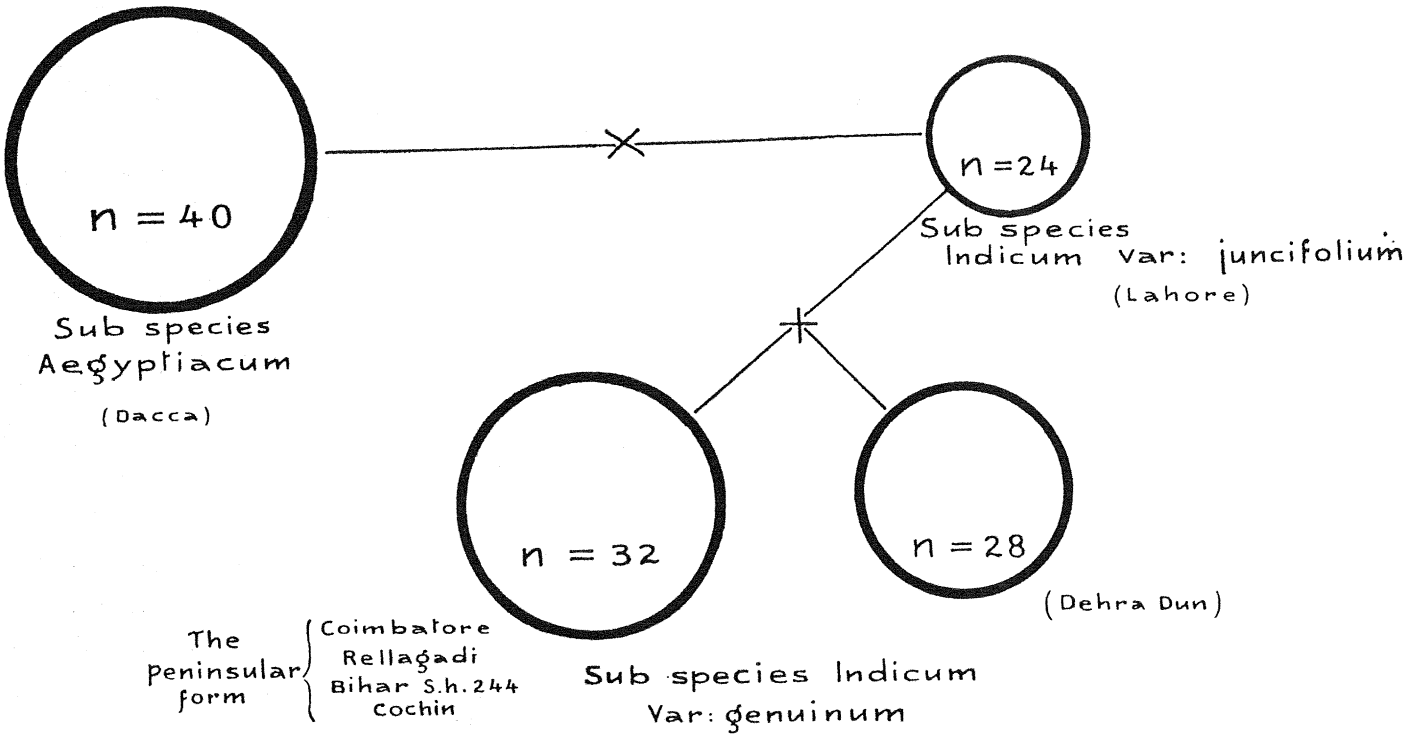
FIG. 14. Metaphase in the Bihar form showing chromosome association.

FIG. 15. Metaphase plate in the Coimbatore form.

FIG. 16. Diakinesis in the Dacca form.

FIG. 17. Metaphase in the Dacca form showing chromosome association.

DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF *SACCHARUM SPONTANEUM* IN INDIA



[Darlington, 1932], the increase in the number of chiasmata observed may be an indication of structural changes in the two chromosomes of a bivalent. The presence of a tetravalent is also noteworthy. Plate III, fig. 12 shows a pollen mother-cell at prometaphase stage. The chromosomes associate in groups of two, three and four which seems to be the prevailing type of secondary association in this variety. The later stages of meiosis are normal.

(c) *The Bihar form (S. H. 244), 2x = 64*

This is another form in which there are thirty-two bivalents in the pollen mother-cell (Plate IV, fig. 13). No tetravalents were observed. Secondary association is more lax than in the Dehra Dun form. Ten groups were differentiated in which at least five were associations of two bivalents (Plate IV, fig. 14).

(d) *The Coimbatore form (Tank), 2n = 64*

Thirty-two bivalents are observed at metaphase (Plate IV, fig. 15). Secondary association of bivalents is of frequent occurrence. They are associated in groups similar to the Bihar form (S. H. 244) and about ten groups could be traced. A number of bivalents with single chiasma, which remain unterminalized, was observed at diakinesis giving a configuration like X.

(e) *The Dacca form 2n = 80*

Plate IV, fig. 16 shows a reconstructed nuclei from two sections of a pollen mother-cell at diakinesis. Variation in length of chromosomes is very marked in this variety. In particular a number of very small chromosomes are found to associate in groups at pro-metaphase. "Secondary association" is very marked (Plate IV, fig. 17) and the groups are composed of two, four and five bivalents. An analysis of the groups shows that in a number of pollen mother-cells the forty bivalents arrange themselves into four groups of five, four groups of four and two groups of two, making in all ten groups.

V. EVIDENCE OF HYBRIDITY WITHIN THE SPECIES

Secondary polyploidy.—It would appear from the chromosome counts of the various clones examined that the species *S. spontaneum* represents a polyploid series. The association of more than two chromosomes at metaphase of meiosis was first observed by Kuwada [1910] in tetraploid *Oryza sativa*. This phenomenon has since been observed in many polyploids. Except for the single tetravalent observed in Rellagaddi, no multivalents have been observed so far in the Indian species. Its absence goes to prove that polyploidy in this species must have occurred through hybridization and not through somatic duplication of chromosome sets, as in auto-polyploids like *Tulipa*.

Besides the formation of multivalents, which is the result of prophase pairing, a secondary or post-synaptic association of bivalents, arising from a general affinity of relatively homologous bivalents, was first observed by Darlington [1926] in Prunus. It is generally observed in plants with small chromosomes. Lawrence [1932] has shown that "secondary association" is of wide occurrence in many species and provides "valuable criterion for homology in hybrid polyploids". We have in *S. spontaneum* strong evidence of this post-synaptic association. The bivalents generally associate into groups of five or ten, which is the basic number of other Andropogonae like *Sorghum*, *Erianthus*, *Imperata* and *S. officinarum*.

In *S. spontaneum* (Dehra Dun) the eighteen bivalents arrange themselves into ten groups as follows:—

Five groups of two bivalents
Two groups of three bivalents
Three groups of four bivalents

There is also an indication of a closer affinity between two of these ten groups so that in some nuclei a more compact association into five groups may be observed. If *A*, *B*, *C*, *D* and *E* represent the basic chromosomes, we can consider the gametes in the Dehra Dun form as being made up approximately of the following chromosome complement:—

1. *A.A.A* + *A.A.A.A.*
2. *B.B.B* + *B.B.B.B.*
3. *C.C* + *C.C.C.C.*
4. *D.D* + *D.D.*
5. *E.E* + *E.E.*

We thus see that the twenty-eight bivalents are composed of two groups of sixteen and twelve bivalents respectively. Since these numbers correspond to the haploid number of the Peninsular forms with thirty-two chromosomes and the Lahore form with forty-eight chromosomes, the Dehra Dun form may be a hybrid between these two chromosome types.

The presence of five pairs of bivalents in the Peninsular form, which stand apart from the rest, seems to indicate that this variety is also a product of hybridization. It is highly probable that the thirty-two chromosome forms have arisen as natural crosses between a type like Dacca ($n = 40$) and a type like Lahore ($n = 24$). In its morphological characters the Peninsular form is found to be intermediate between the wire-leafed "Lahore" and the broad-leafed "Dacca". The range of variation found in this group is also very wide which may be an additional evidence for its being considered a hybrid.

Chiasma behaviour.—The chromosomes of *S. spontaneum* examined fall into two types from the point of chiasma behaviour.

Type I with a single chiasma which is localized.
Type II with one or two chiasmata which are formed at random.

The latter have a fairly high frequency of terminalization.

The presence of bivalents with greater frequency of chiasmata in proportion to their length is noteworthy (Plate III, fig. 6). This is in accordance with the "chiasma theory of pairing" of Darlington. If the frequency of pairing is proportional to length, some of the shorter bivalents would be unpaired at metaphase which would then lead to irregularity in germ formation and thus interfere with sexual reproduction of the species. This property may thus be considered as a genetic factor influencing the behaviour of chromosomes [Darlington, 1932].

This differential behaviour in the chiasma formation of the *S. spontaneum* may be taken as another evidence of its hybridity. This will be dealt with more fully in a separate paper.

VI. Conclusions

Barber, in his "Studies of *Saccharum*" [1918], found the broad-leafed form from Dacca to resemble cultivated canes more than any of the other Indian forms examined by him. This form is now found to have the same number of chromosomes as *Saccharum officinarum*. The discovery of other types with $n = 40$ chromosomes, like *S. robustum* from New Guinea and Glagah Tabongo, from Celebes points to this form having a wide distribution in the tropics. From a study of its morphological characters, the Dacca form comes nearest to Hackel's subspecies, *ægyptiacum*.

It seems that the wire-leafed form from Lahore with $2n = 48$ represents a form of *Saccharum spontaneum* having a basic number six or twelve instead of the usual five or ten found in other Andropogonae. There is strong indication that the forms with thirty-two and twenty-eight chromosomes, which represent the "intermediate" forms of Hackel, are natural hybrids or their back-crosses, between the two types ($n = 40$) and ($n = 24$). These intermediate forms, because of their wide distribution, rightly represent the sub-species '*indicum*', variety "*genuinum*" of Hackel. They show great variation in vegetative characters and are best suited for the study of the differentiation of the species from an ecological point of view. The wire-leafed form, which Hackel describes as subspecies *indicum*, variety *juncifolium*, deserves to be classed as a separate species perhaps "*Saccharum juncifolium*", because of its distinctive morphological characters and its unique chromosome complement.

It is highly probable that the more primitive types of *S. spontaneum* are those with lesser number of chromosomes. These studies on Indian forms

indicate that the primitive forms occur in India. It is likely that a more comprehensive study of the species will lead to the discovery of more "ecotypes" with chromosome number forty-eight and fifty-six than those hitherto examined.

VII. Summary

The Indian types of *S. spontaneum* form a polyploid series with the following chromosome numbers :—

$2n = 48$
$2n = 56$
$2n = 64$
$2n = 80$

2. The somatic chromosomes show primary and secondary constrictions. They also vary in length.

3. Pairing of chromosomes at meiosis is by means of chiasmata. Chromosomes are always associated as bivalents except in Rellagaddi in which a single tetravalent was observed.

4. The number of chiasmata observed is not always proportional to length of chromosomes.

5. "Secondary association" of bivalents is observed. The chromosomes arrange themselves into five or ten groups which is the basic number of the Andropogonae.

6. There is evidence of hybridity within the species. Of the Indian forms examined, the type with forty-eight chromosomes represents Hackel's variety "*juncifolium*" and the type with eighty the variety "*ægyptiacum*". Those with intermediate numbers seem to be natural hybrids between these two forms.

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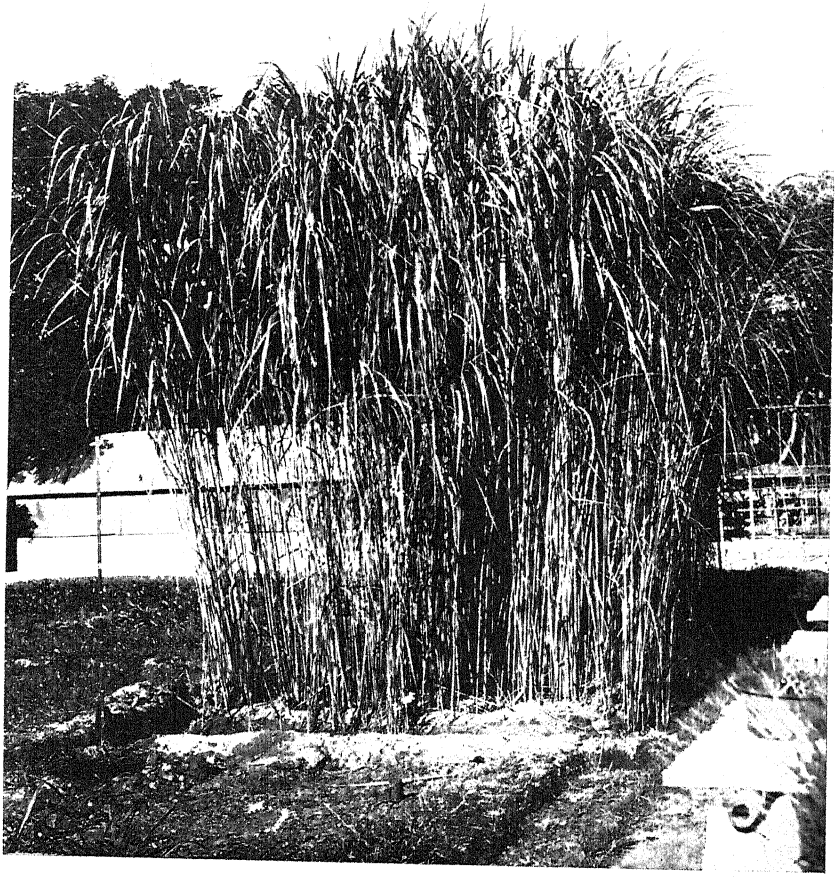

Burma variety of *Saccharum spontaneum*.

CHROMOSOME NUMBERS IN A BURMA *SACCHARUM SPONTANEUM* L. & ITS SEEDLINGS

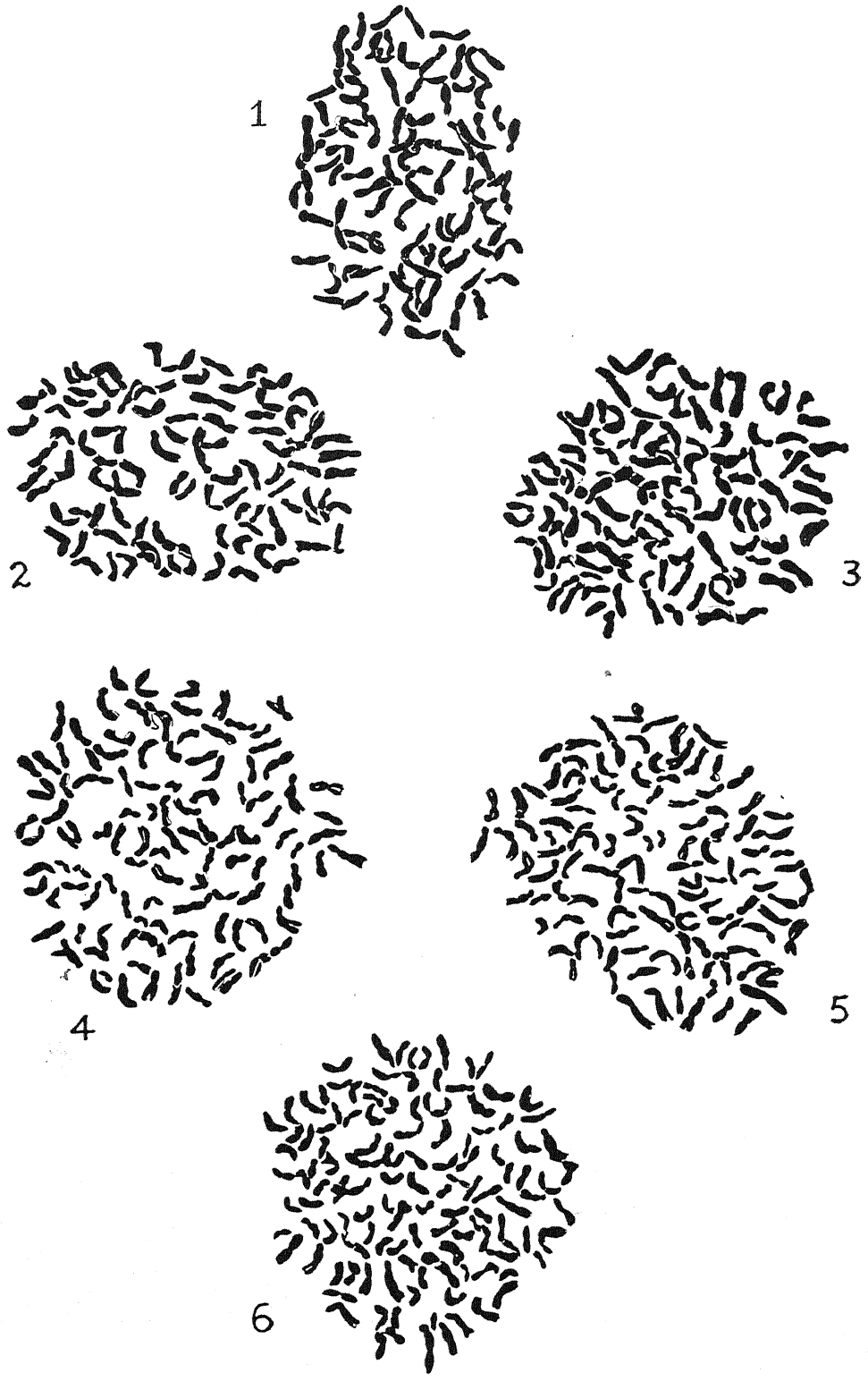

FIG. 1. Somatic metaphase from the root tip of the parent Burma *S. spontaneum* $2n=96$.

FIGS. 2–6. Somatic metaphases from the root tip of some seedlings of the Burma form. $2n=96$; $2n=108$–110; $2n=$

CYTO-GENETIC ANALYSIS OF *SACCHARUM SPONTANEUM* L.

2.—A TYPE FROM BURMA

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(Received for publication on 15th August 1935)

(With Plates VI-VIII)

According to Barber [1918] *Saccharum spontaneum* in Burma represents a series of forms ranging from a thick "Land form" to an aquatic type like the Dacca form of India. The only Burma variety of *S. spontaneum* in the collection at the Imperial Sugarcane Station is from a clone from Mandalay collected in 1929. This variety is the most outstanding of the *S. spontaneums* grown at Coimbatore. It attains a height of fifteen to eighteen feet, propagates very profusely by means of underground stems and has a splendid erect habit (Plate VI). The stem has a much longer internode than any of the other *S. spontaneums*. Panje [1932] notes a number of other morphological characters in which this form differs from the Indian and the East Indies types studied by him. The plant flowers very rarely in Coimbatore and only a single inflorescence was observed in 1933.

Cytological observation.—As the plant did not arrow in 1934-35, only root tips were available. These were fixed in Allen's modification of Bouin's PFA-15b after freezing, sections cut at 10μ and stained with Iron Alum haematoxylin with picric acid as de-stainer. The metaphase plate of somatic division showed ninety-six chromosomes (Plate VII, fig. 1). This is the first record of this number for any type of *S. spontaneum* studied. The chromosomes appeared similar to those of the Indian *S. spontaneum* studied, in having both primary and secondary constrictions. The prevalence in India of forms of *S. spontaneum* with chromosome $2n=64$ [Dutt and Rao, 1933 and Singh, 1934] and the occurrence in Sumatra of a "gigas" form with $2n=124$ [Singh, 1934] points to the Burma form being a triploid with basic chromosome number $x=32$.

Triploids occur in nature either as the result of a cross between diploids and tetraploids of the same species or as the result of the failure of reduction in the archesporium of one of two diploid parents and the consequent fusion of an x with an unreduced $2x$ germ cell. According to Bremer [1929] doubling of chromosomes in the female parent is a common phenomenon in *Saccharum*. The regionally intermediate Burma form might be either a natural cross between an Indian form with $n=32$ and a tetraploid form like the "gigas" form of Sumatra (Plate VIII) or it may represent the product of fertilization of an abnormal diploid gamete with a haploid one as noted by Bremer in many other *Saccharum* species. Cytological evidence from pollen mother-cell studies is necessary to throw further light on the origin of this interesting Burma form. Its possible position amongst other *S. spontaneum* is shown in the diagram.

Progeney of the Burma form.—The single arrow produced in 1933—and left unbagged—yielded a number of seedlings which showed marked differences in morphological characters. Many of these were thicker than the parent and looked like very medium sugarcanes. Root tips of some of these were examined and they showed 96 to 124 chromosomes at somatic metaphase (Plate VII, figs. 2-6). As the arrow was unbagged, and consequent possibility of cross-pollination, much value cannot be placed on the variation observed in their chromosome numbers. However the numbers found fall within the expected frequency of chromosome distribution in a triploid with $2n=96$.

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DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF *SACCHARUM SPONTANEUM*

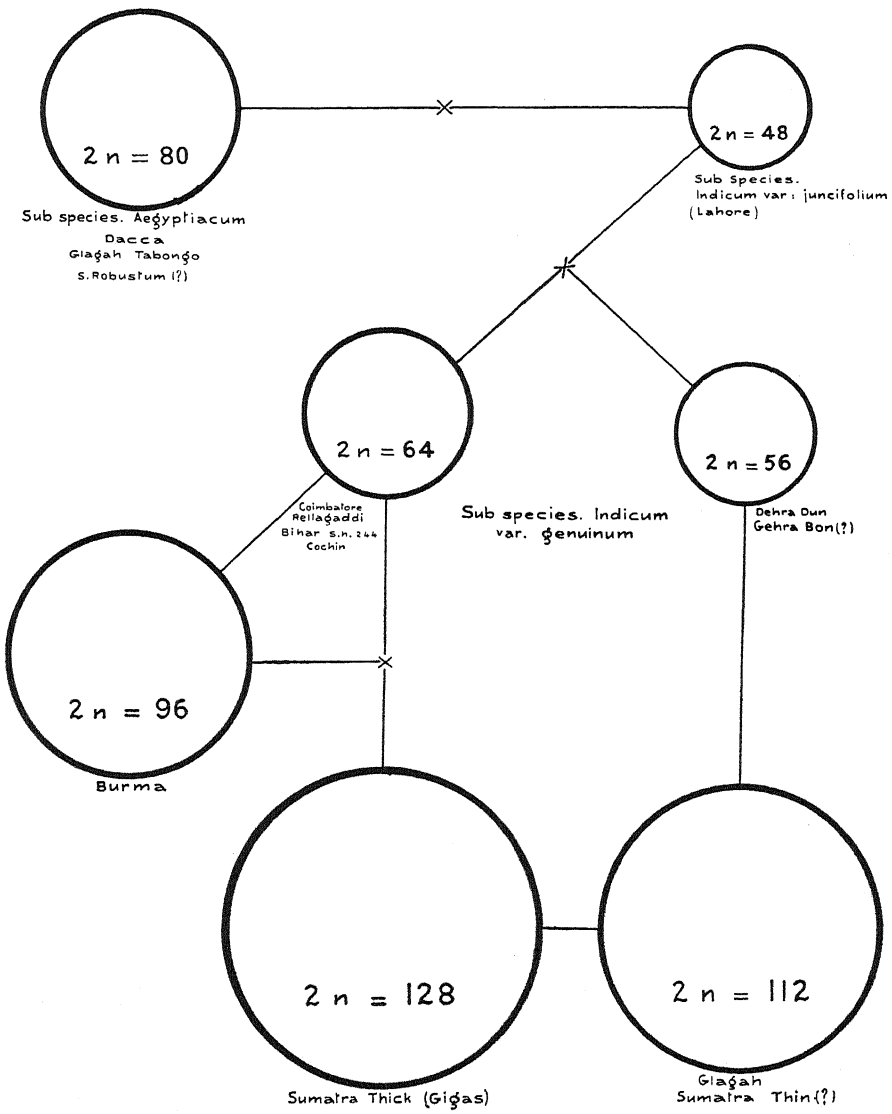

FIG. 1. Healthy seedling.
FIGS. 2-5. Seedlings showing various stages of the disease.
FIG. 6. Cane leaf showing diffused patches.
FIG. 7. Wheat leaf showing definite spot formation.
FIG. 8. Barley leaf showing closely arranged stripes.

SOME NEW SEEDLING DISEASES OF SUGARCANE

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(With Plates IX-XI)

During March 1934 sugarcane seedlings growing in pans at the Cane Breeding Station at Coimbatore were found to die in large numbers. The disease was especially widespread in the thick cane seedlings. The first indication of the disease in the pans was the appearance of a number of seedlings wilting or dying in small patches. Sometimes only one or two pans in the same culture showed the disease whilst others remained free. In the dying seedlings root development was very poor, and in some cases the roots were very soft, rotten and discoloured. In a few cases the roots had developed well, but the tissues round the collar showed brownish discoloration and the leaf tips were drying downwards. Sometimes the collar had simply become soft without any brownish discoloration. A species of *Helminthosporium* was found growing profusely in the dead seedlings.

In January 1935 all the new sowings were again examined and the following fungi were found in the dying seedlings. Two species of *Helminthosporium*, *Pythium* sp., *Fusarium* sp., *Alternaria* sp., *Nigrospora* sp., and a sclerotial fungus. *Helminthosporium* spp. and *Pythium* sp. were most prominent. The present paper deals with two species of *Helminthosporium* and a *Pythium* sp. which were found to be parasitic.

I. *Helminthosporium* SPP.

Symptoms.—The disease usually appears in the early stages of the growth of the seedlings, forming small, narrow, reddish stripes, crowded together but not forming definite spots with a margin. When older they are bright to dark brown in colour. The leaf-sheaths become dark brown or olivaceous owing to the formation of numerous conidiophores and conidia.

Morphology.—The mycelium consists of septate, subhyaline hyphae, both inter and intra-cellular, which ramify in the tissues. Haustoria have not been found. The hyphae are 2 to 4μ in breadth. Conidiophores are formed in the discoloured portion of the leaf-sheath and emerge through the epidermal cells

singly or in pairs. They are unbranched, brown to dark olivaceous, 2 to 5 septate, 40 to 130μ in length and 5 to 7μ in breadth. The basal cell of the conidiophore is slightly swollen. The geniculation associated with the production of successive conidia is well defined. Conidia are borne singly at the tip of the conidiophores, and as successive ones are being formed the tips become curved owing to the large number of geniculations, size 20-140·5μ × 9-16·5μ, average 62 × 13·5μ 3 to 11 septate not constricted at the septum. The end septa are deeply marked; spores are dark brown, end cells lighter in colour, tapering towards both ends with a prominent hilum. They germinate by producing germ tubes from either end cell and sometimes from the intermediate cells also.

The following inoculation experiments were carried out to test pathogenicity.

TABLE I

Results of experiments carried out to test the pathogenicity

Date	Host	Place of inoculation	Result	Control
24th May 1934 .	Co. 213 cane .	4 shoots inoculated in different places.	+	Free
29th May 1934 .	Co. 213 cane .	8 shoots, leaves . .	+	Do.
28th June 1934 .	Co. 213, 416, 419, 420, M16, B3412 Lalgirah cane.	Plants in pots. Leaves inoculated in different places. Plants moist by spraying with water.	+	Do.
25th Feb. 1935 .	Wheat . .	30 seedlings ; leaves . .	+	Do.
	Barley . .	30 seedlings ; leaves . .	+	Do.
14th May 1935 .	Maize . .	6 plants ; leaves . .	+	Do.
	Jowar . .	6 plants ; leaves . .	+	Do.
	Co. 213 cane .	6 shoots ; leaves . .	+	Do.
31st May 1935 .	Co. 213 cane .	6 canes, leaf and leaf-sheaths in different places.	+	Do.

This species gives a very high percentage of infection not only with different varieties of cane but also with maize, *jowar*, wheat and barley. On wheat it forms definite spots with a grey centre and reddish brown margin similar to those of *Helminthosporium sativum* P. K. & B.

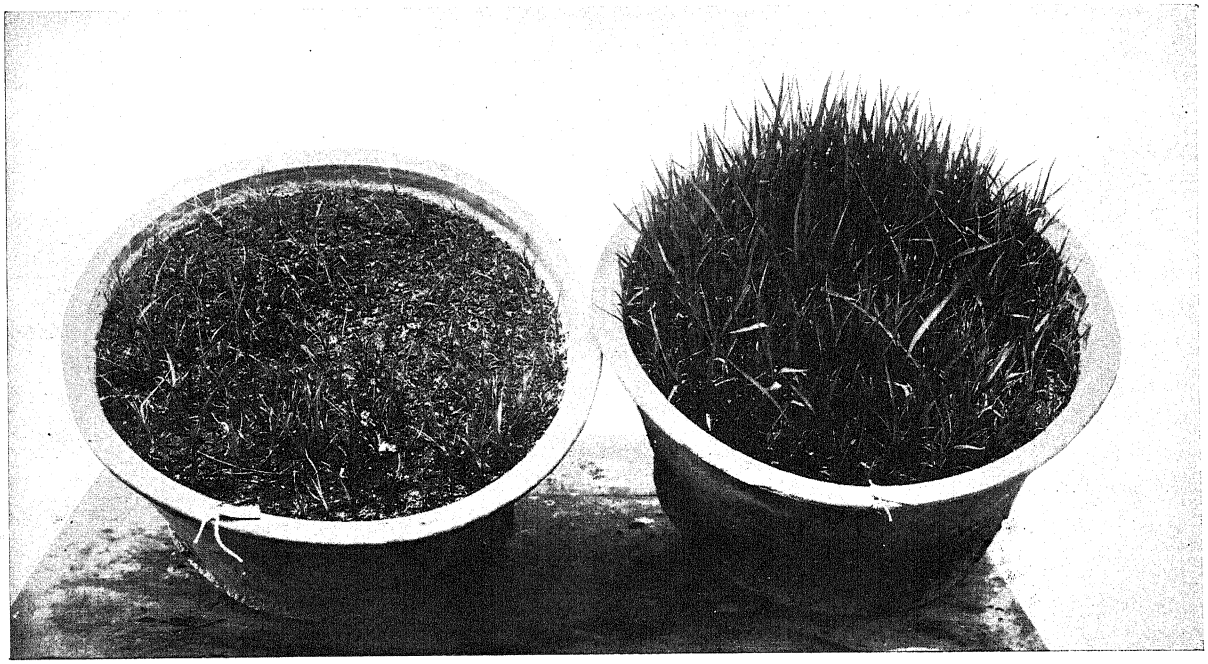

FIG. 1. Diseased and healthy seedlings.

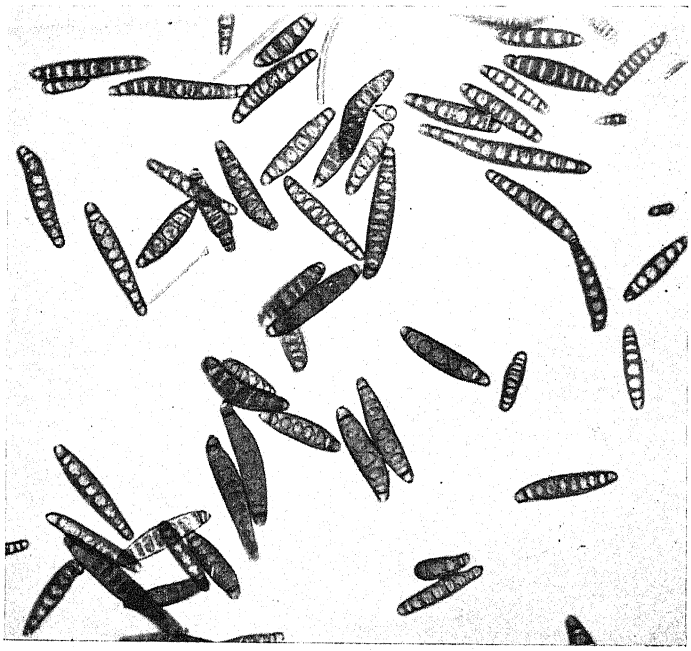

FIG. 2. Conidia of *Helminthosporium halodes* (×250).

The disease has so far been observed only in the seedlings. When selected seedlings from diseased pans are transplanted in the field, they do not show any symptoms of the disease.

Identity of the fungus.—The measurements of this fungus together with the degree of septation and shape of spores agree very closely with that of *H. halodes* described by Drechsler [1923] on the leaves of *Distichlis spicata* (L.) Greene, from New York. Mitra [1931] described *H. halodes* var. *tritici* on wheat from Central Provinces. He succeeded in infecting leaves of maize, *jowar*, wheat, barley, oats, rice, and cane with this strain. The measurements of these three fungi are given below for comparison :—

TABLE II

Measurements of the fungi

Fungus	Spore length	Spore width	Septa
H. halodes	20-103μ	10-14μ	1-12
H. halodes var. *tritici*	23-73μ	13-20μ	2-9
Isolation from cane seedlings	20-104·5μ	9-16·5μ	3-11

It will be seen that the cane strain of *Helminthosporium* agrees in size and septation of the spores more closely with *H. halodes* than with *H. halodes* var. *tritici*.

This is the first record of its occurrence on sugarcane.

In addition to *H. halodes* another species of *Helminthosporium* has been isolated from sugarcane seedlings which agrees with the description of *Helminthosporium tetramera* described by MacKinney [1925] from Oklahoma, U. S. A., where it is found associated with foot-rots of wheat. Infection experiments carried out proved that this fungus is a weak parasite when compared to *H. halodes*.

II. *Pythium* SP.

The roots of cane seedlings become soft and rotted, the collar flaccid and discoloured and the seedlings collapse near the ground level. When seedlings are pulled out the rotten roots are left behind in the soil.

Infection experiments carried out to prove its pathogenicity are summarised below :—

TABLE III

Results of infection experiments

Date	Plant	Where inoculated	Infection	Control	Remarks
6th July 1935	Sugarcane .	5 seedlings in water culture ; inoculated at roots.	+	Free	
	Maize . .	Do. .	+	Do.	Slight infection.
	Jowar . .	Do. .	+	Do.	Do.
27th July 1935	*Carica papaya* .	Fruits . .	—	Do.	Fungus entered through wound in *Carica papaya* and *Lagenaria vulgaris*, but infection did not spread.
	Hibiscus esculentus.	Do. . .	—	Do.	
	Trichosanthes dioica.	Do. . .	—	Do.	
	Lagenaria vulgaris.	Do. . .	—	Do.	
	Amaranthus sp.	Stem . . .	—	Do.	
3rd Aug. 1935	Cane, maize, *jowar.*	6 seedlings each, in water culture ; kept at 25°C.	+	Do.	
5th Aug. 1935	Wheat and barley.	40 seedlings each, in sand culture ; kept at 25°C. infected at collar.	+	Do.	Very active infection, collar becomes soft, brown and collapses.
6th Aug. 1935	Maize . .	6 seedlings growing in sand ; infected at the collar.	+	Do.	Slight infection.
	Jowar . .	Do. . .	+	Do.	Do.
13th Aug. 1935	Sugarcane .	3 shoots in pots ; culture placed touching the leaf-sheath.	+	Do.	
	Maize . .	Roots floating in water ; culture placed touching the roots.	+	Do.	Very slight infection.
16th Aug. 1935	Wheat . .	40 seedlings in sand ; culture placed at collar ; kept at 25°C.	+	Do.	Very active infection.
2nd Sept. 1935	Wheat, barley and oats.	20 seedlings each.	+	Do.	Do.

FIG. 1. Conidia of *Helminthosporium tetramera* (×250).

FIG. 2. Conidia and oospores of *Pythium graminicolum* (×250).

FIG. 3. Branched antheridia and oogonia of *Pythium graminicolum* (×500).

When cross-inoculated, the fungus attacks maize, *jowar*, wheat, oats and barley in addition to sugarcane. On maize and *jowar* it is not very active but on wheat, oats and barley seedlings it is very vigorous and kills the seedlings in three days. The collar and the roots become soft and discoloured brown or yellowish brown. The mycelium is found in all the tissues. Later on oospores develop in large numbers in the epidermal tissues.

The measurements of sporangia, zoospores and oospores of the strain from cane seedlings agree in all respects with those of *Pythium graminicolum* described by Subramaniam [1928] on wheat from Dharwar, Bombay. Matthews [1931] isolated *Pythium graminicolum* from a sandy soil in North Carolina in which Sarracenia was growing.

The measurements of the three strains are given below for comparison :—

TABLE IV

Measurements of the strains

Fungus	Host	Oogonia	Average	Oospores,	Average
Pythium graminicolum.	Wheat .	16·5-28·6μ	..	15·4-16·4μ	
Do. .	Sarracenia	24-36μ	30μ	21·6-32μ	28μ
Do. .	Sugarcane	16·9-33μ	25μ	16·3-10·8μ	23μ

This is the first record of *Pythium graminicolum* on cane seedlings in India.

III. CONTROL MEASURES

The seeds of the susceptible variety P. O. J. 2725 × Co. 243 were obtained from Coimbatore and sown at Pusa, where the disease also developed. It is therefore presumed that the foot-rot disease is seed-borne. Treatment of the seeds and soil with a solution of 0·25 per cent Uspulun checked the disease to a large extent both at Coimbatore and Pusa, very few seedlings dying in the treated pans. When the diseased seedlings were removed and the rest were allowed to grow, they remained healthy throughout the period they were kept under observation.

So far the disease has been observed only on very young seedlings. Should it become a serious source of inconvenience, it can be controlled by application of suitable fungicides to seed and soil.

SUMMARY

Helminthosporium halodes, and *H. tetramera* have been found to be the cause of seedling diseases of sugarcane. *H. halodes* is an active parasite, while *H. tetramera* is a weak one. These two fungi cause foot-rot of the seedlings, and are here recorded for the first time on sugarcane.

Pythium graminicolum has been found on sugarcane for the first time in India as the cause of a seedling disease. This fungus when cross-inoculated attacks cane, wheat, oats and barley very vigorously and maize and *jowar* to a slight extent.

ACKNOWLEDGMENTS

The writer is indebted to the Imperial Council of Agricultural Research under whose scheme for research on "Mosaic and other diseases of sugarcane" the work has been carried out. His thanks are also due to Dr. M. Mitra, Assistant Mycologist, for the microphotographs and to the officers of the Cane Breeding Station, Coimbatore, for facilities in connection with this investigation.

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A BIOCHEMICAL STUDY ON THE DECOMPOSITION OF FARMYARD MANURE AND SULPHATE OF AMMONIA IN THE PADDY SOILS OF THE CENTRAL PROVINCES*

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INTRODUCTION

The decomposition of sulphate of ammonia or farmyard manure in different soils under normal arable conditions has been widely studied ; but comparatively little is known regarding the chemical and microbiological processes involved in the decomposition of such fertilisers under the usual water-logged conditions of the paddy field.

There are some scattered references in the literature to nitrogen changes in submerged soils due to the addition of nitrogenous fertilisers. Warington [1892] for the first time detected in some laboratory experiments a loss in added nitrates when the soils were kept water-logged. Daikuhara and Imasaki [1907] also made similar observations. Nagaoka [1904] was the first to report the formation of nitrite when nitrates were added to paddy fields and Kelley [1911] stated that nitrites so formed were toxic to paddy seedlings. In a recent paper Kapp [1933], however, observed that in Arkansas soils application of sodium nitrate and ammonium sulphate does not lead to accumulation of nitrite in submerged rice soils in sufficient quantities to prove toxic to the seedlings. According to Oelsner [1918] nitrates as well as total nitrogen are lost on water-logging the soil. Subrahmanyan [1927] failed to observe any such diminution in total nitrogen although he noticed that in submerged soils there was a slight diminution in nitric nitrogen and a distinct increase in free and saline ammonia. According to Harrison and Aiyer [1913-14] nitrification is impossible in sub-merged paddy soil and the nitrates produced during the dry season are quickly denitrified so that the nitrogen required by the crop is obtained from the ammonia and nitrogenous organic compounds produced by the anaerobic decomposition of the proteins of added green manure.

* The investigation recorded in this paper was carried out at the Bio-Chemical Laboratory of the Central Provinces Rice Research Scheme financed by the Imperial Council of Agricultural Research.

The work described in this paper was mainly undertaken with the objects of ascertaining firstly, the relative effect of the decomposition of farmyard manure and sulphate of ammonia under water-logged conditions on the nitrogen contents and microflora of two of the typical rice soils of the Central Provinces and secondly, to what extent the results vary fundamentally from those obtained under aerobic soil conditions.

Experimental

The soils used for these experiments were "*Matasi*" (sandy loam) and "*Dorsa*" (medium clay) from unmanured rice plots on the Government Experimental Farm at Labhandi, Raipur. Geologically, these soils are formed entirely upon Pre-Cambrian rocks of the Purana and Archaean groups. Their chemical and mechanical compositions are given below.

Table I

Chemical and mechanical compositions of Matasi and Dorsa soils

(*a*) *Chemical analysis*

	Matasi	*Dorsa*
	Per cent	Per cent
Insoluble silicate and sand	87·41	77·78
Alumina	4·74	11·43
Lime	0·62	0·39
Potash	0·46	0·86
Phosphoric Acid	0·02	0·03
Magnesia	0·30	0·81
Loss on ignition	6·70	7·80
Carbon	0·56	0·56
Total	100·81	99·66
Total nitrogen	0·05	0·06
Available phosphoric acid	0·001	0·001
Available potash	0·10	0·11
pH value	6·8	7·2
C/N	11·2	9·3

(*b*) *Mechanical analysis* (*By Puri's method*)

Soil	Clay	Fine silt	Coarse silt	Sand	Total
	Per cent	Per cent	Per cent	Per cent	
Matasi	25·36	3·70	8·84	58·74	96·64
Dorsa	48·26	5·08	7·86	34·86	96·06

A series of comparative tests on the changes in bacterial numbers, ammonia, nitrate and nitrite contents, carbon dioxide evolution and nitrate-reducing capacity in culture solutions were carried out in each of these soils receiving farmyard manure (well-rotted cattle-dung), sulphate of ammonia or no nitrogen under water-logged and aerobic conditions.

The principle of the technique employed is the same as communicated in a preceding paper by one of the writers [Mukerji, 1932] for following the biochemical changes in soils under conditions approximating more closely to those of the field than are obtained in the conventional tumbler technique or in pot cultures. Air-dried samples of soils (0—6 in.) were passed through one-mm. sieve, and 1000-grm. lots uniformly moistened to fifty per cent of the water-holding capacity of the soil by applying as a spray about 200 c.c. of water. In a parallel series the soils were maintained under water-logged conditions by having a film of water always standing over the surface. Wide-mouthed glass jars (two-litre capacity) with one kg. of soil were set up under the two different moisture conditions with soil receiving farmyard manure, sulphate of ammonia or no nitrogen. The manures were applied at the rate of 100 lb. nitrogen per acre and their doses per kg. of soil calculated on the basis of one acre of soil weighing approximately two million lbs.

For each treatment two jars were set apart. The lower ends of the jars were cut off from light by dark paper shades. Aerobic and submerged cultures were sampled for analysis on alternate days. The sampling which was done by boring with a small augur involved thorough mixing of the soils and constant renewal of the air over the soil mass. The jars were shaken every day for a few minutes to provide free exchange of gas between the soil particles, supernatant water (in water-logged cultures) and the air above the soil.

Soil samples were withdrawn from each individual jar every week, and bacterial numbers, ammonia, nitrate and nitrite determined. Matthews' aeration method was used for the determination of ammonia. Nitrate and nitrite were estimated by Harper's phenol-disulphonic acid method and Griess-Ilosva method, respectively. Bacterial counts were made by plating on Thornton's agar medium. Besides ammonifying organisms this medium is also capable of growing rapidly, as has been reported in a previous publication by one of the authors [Mukerji, 1931], some species of nitrite forming bacteria which differ widely from *Nitrosomonas* or *Nitrosococcus*.

Bacterial numbers and nitrogen changes

The bacterial counts are given below.

Table II

Average bacterial numbers in millions per grm. of soil

(The means of the replicate counts for each treatment have been assigned with their respective probable errors.)

Treatments	*Dorsa* soil							*Matasi* soil					
	Days from commencement							Days from commencement					
	0	7	14	28	42	49	56	0	7	14	21	28	35
Farmyard manure—													
AE	15·0 ‡2·7	10·1 ‡1·8	24·2 ‡2·0	9·4 ‡1·9	9·4 ‡1·8	19·6 ‡2·1	18·4 ‡2·2	22·9 ‡1·3	26·8 ‡1·8	25·1 ‡2·0	21·9 ‡0·9	14·3 ‡2·1	10·7 ‡1·0
WL	15·0 ‡2·9	11·9 ‡0·9	16·8 ‡1·9	5·4 ‡1·0	1·2 ‡0·4	6·0 ‡0·5	6·1 ‡1·9	22·9 ‡1·5	18·6 ‡2·1	15·3 ‡1·4	10·1 ‡2·3	4·0 ‡0·6	7·4 ‡2·1
Ammonium sulphate													
AE	13·8 ‡0·9	19·1 ‡2·2	15·1 ‡0·7	5·9 ‡1·3	22·3 ‡1·6	33·6 ‡2·1	27·7 ‡1·6	3·8 ‡0·5	29·5 ‡2·1	19·3 ‡1·3	15·8 ‡1·1	11·1 ‡0·9	14·3 ‡1·2
WL	13·8 ‡1·6	16·2 ‡2·1	8·6 ‡0·7	6·5 ‡0·7	3·0 ‡0·4	7·9 ‡2·6	5·8 ‡1·9	3·8 ‡0·7	20·0 ‡1·6	17·7 ‡1·0	14·7 ‡1·0	6·8 ‡0·4	11·8 ‡1·5
No nitrogen—													
AE	16·3 ‡2·1	38·3 ‡1·2	31·5 ‡1·0	13·6 ‡2·4	18·9 ‡2·2	28·9 ‡0·8	33·1 ‡1·9	9·8 ‡1·2	44·9 ‡3·1	16·7 ‡0·8	17·6 ‡1·7	7·8 ‡0·9	8·9 ‡0·8
WL	16·3 ‡1·0	14·7 ‡1·7	15·9 ‡2·3	5·3 ‡1·9	11·4 ‡2·1	9·5 ‡1·4	6·8 ‡0·8	9·8 ‡1·4	34·5 ‡3·0	16·7 ‡1·0	10·1 ‡0·7	5·1 ‡0·7	4·1 ‡0·8

AE = Aerobic.
WL = Water-logged.

It will be observed that in *Matasi* soil, both under aerobic and water-logged conditions, the general level of the bacterial population with sulphate of ammonia and no nitrogen was low at the commencement but rose remarkably during the first week; the numbers subsequently decreased with minor fluctuations. With farmyard manure the population was maintained at a uniform high level from the very beginning to the end of the third week, afterwards there was a gradual decrease. No such uniform change was noticed in *Dorsa* soil in which the bacterial numbers, although fairly high at the outset, showed only periodic rise and fall. The bacterial numbers in the farmyard manure and sulphate of ammonia treated cultures of this soil were distinctly lower than in the control. This was confirmed, as shown later, by the respective amounts of carbon dioxide evolved from *Dorsa* soil receiving all these treatments. The depressing effect of farmyard manure on the bacterial population in the heavier of the two soils is somewhat surprising, and is apparently due to the formation of some intermediate product which proves toxic to the organisms capable of growing on Thornton's medium.

The means of five or more counts at seven-day intervals for the whole experiment are given below.

TABLE III

Means of bacterial numbers at seven-day periods

Treatments		*Dorsa* soil (No. in millions per grm. of soil)	*Matasi* soil (No. in millions per grm. of soil)
Farmyard manure	Aerobic	15·2	20·3
	Water-logged	8·9	14·1
Ammonium sulphate	Aerobic	19·6	15·6
	Water-logged	8·8	12·5
No nitrogen	Aerobic	26·2	16·1
	Water-logged	13·4	11·4

It is obvious that the relative effect of the individual manurial treatment on the bacterial population is not the same in the two soils.

On comparing the aerobic cultures among themselves it is found that farmyard manure gave in general the highest bacterial counts in *Matasi* soil and the lowest in the heavier soil—*Dorsa*. Sulphate of ammonia also depressed the population very markedly in *Dorsa* soil, whereas in *Matasi* soil it did not bring about any appreciable change in the bacterial numbers.

Under water-logged condition *Matasi* soil did not show any marked difference in bacterial counts between the farmyard manure, sulphate of ammonia and no nitrogen treatments. But in *Dorsa* soil under similar condition the numbers in the farmyard manure or sulphate of ammonia treatment were distinctly less than those in the control.

The water-logged cultures in both the soils gave consistently lower numbers than the aerobic ones. This is according to expectation, since the organisms counted in the present case were mostly aerobic. One important fact that emerges out of the plate counts is that strictly aerobic bacteria are present in surprisingly large numbers under anaerobic condition in water-logged soils—thus doubtlessly proving the presence of a fairly abundant supply of oxygen in the rice soils during the crop season.

Table IV gives for each treatment the mean differences in bacterial numbers between the aerobic and water-logged cultures.

TABLE IV

Mean differences in bacterial numbers between aerobic and submerged cultures

Treatments	*Dorsa* soil (millions per grm. soil)	*Matasi* soil (millions per grm. soil)
Farmyard manure	6·3	10·4
Ammonium sulphate	10·8	3·5
No nitrogen	14·9	4·6

It is interesting to note that both sulphate of ammonia and no nitrogen water-logged treatments depressed the bacterial population as determined by the plate counts more in *Dorsa* soil than in *Matasi*. Farmyard manure, on the contrary, caused the least difference in bacterial numbers between the aerobic and water-logged cultures in *Dorsa* soil and the highest in *Matasi*.

The results of ammonia determination in both the series are detailed in Table V.

TABLE V

Ammoniacal nitrogen changes (Ammonia-N as parts per million)

Treatments		*Dorsu* soil									*Matasi* soil					
		Days from commencement									Days from commencement					
		0	7	14	21	28	35	42	49	56	0	7	14	21	28	35
Ammonium sulphate	AE	80·6	79·4	46·8	61·9	86·6	102·1	65·9	39·8	49·3	141·5	80·0	119·0	87·2	91·1	82·4
	WL	80·6	58·3	74·4	74·5	64·8	59·0	75·7	45·7	52·6	141·5	69·0	72·5	55·1	84·3	112·1
Farmyard manure	AE	48·2	32·3	96·1	22·8	45·9	125·2	48·4	47·7	32·8	105·5	72·7	29·4	85·4	66·0	90·2
	WL	48·2	20·7	51·5	57·8	51·2	78·7	53·2	35·5	35·4	105·5	51·4	57·0	46·1	56·3	108·6
No nitrogen	AE	33·6	30·1	31·1	53·3	57·9	51·0	46·0	43·7	48·6	90·9	83·0	99·1	97·2	88·4	141·0
	WL	33·6	19·9	31·1	51·1	59·1	54·1	62·6	42·1	44·8	90·9	68·7	83·0	141·2	48·7	104·1

AE = Aerobic.
WL = Water-logged

Proper precautions were taken in using Mathews' method for these determinations. A water pump was used for aeration; acid sprays were guarded against and much care was taken to ensure that the optimum amount of air passed through the aerating tubes.

It may be observed from the data given above that in *Matasi* soil water-logging led to a diminution in ammonia both in the sulphate of ammonia and no nitrogen treatments. A similar effect, although not so marked, is also noticeable in the case of farmyard manure treatment. In *Dorsa* soil while there was no appreciable change in ammonia as a result of water-logging in culture receiving either farmyard manure or no nitrogen, a slight increase in ammonia took place in the one to which sulphate of ammonia was applied.

The rate of decomposition of farmyard manure under submerged condition approximates to that under aerobic condition—more in *Dorsa* than in *Matasi* soil. The results of the bacterial counts also lead to the same conclusion.

The figures for the accumulation of ammonia in the sulphate of ammonia treatments indicate that in *Matasi* soil the reduction of ammonia under submerged condition is more rapid than its oxidation in the aerobic culture. The reverse is, however, the case in the clayey soil—*Dorsa* in which the effect of the activity of the nitrifying bacteria in the aerobic culture is more pronounced than that of the de-nitrifying organisms under water-logged condition.

It is evident that there is approximately an inverse relationship between bacterial numbers and ammonia. An increase in the bacterial population is accompained by a decrease in ammonia and *vice versa*. This relationship obviously reflects decreased bacterial activity due to increase in numbers.

The results of nitrite and nitrate determinations are shown in Table VI.

TABLE VI

Showing nitrite and nitrate formation

(a) *Nitrite-nitrogen as parts per million*

Treatments		*Dorsa* soil									*Matasi* soil					
		Days from commencement									Days from commencement					
		0	7	14	21	28	35	42	49	56	0	7	14	21	28	35
Ammonium sulphate	AE	0·91	1·70	0·40	0·29	0·41	0·59	0·51	0·19	0·16	0·17	0·09	0·32	Slight	Slight	0·14
	WL	0·91	0·32	0·98	1·21	0·56	*Nil*	0·19	Slight	Slight	0·17	Slight	*Nil*	0·02	*Nil*	0·39
Farmyard manure	AE	0·43	0·37	0·33	0·37	0·41	0·58	0·87	0·23	0·11	Slight	0·61	0·11	0·13	0·14	0·10
	WL	0·43	0·36	0·42	0·34	0·23	0·34	0·41	0·15	Slight	Do.	*Nil*	0·09	0·14	0·19	0·67
No nitrogen	AE	0·76	0·17	0·36	0·51	0·46	0·32	0·44	0·31	0·19	0·17	0·75	0·31	0·15	0·39	0·31
	WL	0·76	0·40	0·50	0·39	0·68	0·31	*Nil*	0·30	Slight	0·17	*Nil*	Slight	0·17	0·18	0·31

AE = Aerobic.
WL = Water-logged.

(b) *Nitrate-nitrogen in parts per million*

Treatments		*Dorsa* soil									*Matasi* soil					
		Days from commencement									Days from commencement					
		0	7	14	21	28	35	42	49	56	0	7	14	21	28	35
Ammonium sulphate	AE	110·4	108·7	102·5	105·4	128·7	180·3	127·2	137·6	117·5	18·7	15·6	31·3	22·4	33·5	30·6
	WL	110·4	82·4	20·4	12·4	Slight	5·5	*Nil*	*Nil*	*Nil*	18·7	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Farmyard manure	AE	96·0	92·6	119·6	81·7	118·9	90·1	93·2	88·8	109·4	19·0	30·6	23·4	61·5	40·9	36·5
	WL	96·0	106·5	19·8	11·1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	19·0	*Nil*	*Nil*	*Nil*	*Nil*	2·9
No nitrogen	AE	92·0	90·6	88·9	72·0	82·8	105·1	111·1	102·7	92·7	17·1	21·8	25·1	39·7	30·6	33·4
	WL	92·0	56·9	38·8	16·8	39·5	Slight	5·9	6·8	Slight	17·1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*

AE = Aerobic.
WL = Water-logged.

There was greater accumulation of nitrite in *Dorsa* than in *Matasi* soil. In either soil the quantity of nitrite in the aerobic cultures exceeded, on the average, that in the corresponding water-logged cultures. It may also be noted that either very little or no nitrate was formed in the submerged cultures.

Since nitrite cannot be detected in sufficient quantities in soils covered with water, it may be concluded that some gaseous nitrogen is given off as the result of the activity of the de-nitrifying organisms. It is probable that the predominance of these organisms in submerged soils serves a very useful purpose in promoting the supply of oxygen to the roots of the rice plant through the reduction of nitrate. Harrison and Aiyer [1913-14] also concluded that the oxygen evolved at the surface of the submerged paddy soil is utilised by the rice plant for the purpose of aeration of the roots. These authors, however, trace the evolution of oxygen to a film of organized growth spread over the surface of the soil. It is also interesting to observe that according to Barritt [1933] de-nitrification occurring in percolating sewage filters accelerates to a very marked extent the oxidation of the organic matter in the sewage.

Reduction of nitrate in submerged soils

The experiments described below were carried out in order to ascertain fully the fate of nitrate added to these soils and gain direct evidence regarding the loss of nitrate under water-logged condition.

Aerobic (moistened to fifty per cent of the water-holding capacity of the soil) and water-logged cultures, similar to those already described, were set up with the same samples of *Dorsa* and *Matasi* soils used in the previous experiments. To each sodium nitrate was added at the rate of 0·05 grm. nitrogen per kg. of dry soil. Soil samples were withdrawn daily and analysed for nitrate, nitrite and ammonia contents.

The daily changes in nitrate-nitrogen are shown in Table VII.

TABLE VII

Reduction in added nitrogen

Days from start	*Dorsa* soil		*Matasi* soil	
	Nitrate nitrogen as parts per million		Nitrate nitrogen as parts per million	
	Aerobic	Water-logged	Aerobic	Water-logged
0	95·00	95·00	67·05	67·05
1	..	..	80·87	34·10
2	90·03	76·01	80·62	49·20
3	89·90	64·25	79·04	50·50
4	104·15	47·34	73·60	48·80
5	96·60	47·62	76·49	49·30
6	96·34	39·30	73·80	49·50
7	103·30	35·17	73·80	29·30
8	102·60	28·04	72·02	42·60
9	95·70	22·50	64·09	31·20
10	88·09	22·70	61·42	25·70

It is obvious that a considerable portion of nitrate disappears very quickly on water-logging the soils—the rate of diminution being much greater in the heavier soil than in the lighter one. In neither of the soils was there any marked disappearance of nitrate under aerobic conditions during the first ter days; whereas, during the same period under water-logged condition the losses registered by *Dorsa* and *Matasi* soils amounted, respectively, to as much as seventy-seven per cent and sixty-two per cent of the quantity originally added. It is therefore evident that the nitrate-reducing bacteria are more active in *Dorsa* than in *Matasi* soil.

The nitrite and ammonia figures are given below.

TABLE VIII

Showing accumulation of nitrite and ammoniacal nitrogen in soil receiving sodium nitrate

Days from start	*Dorsa* soil Nitrogen as parts per million				*Matasi* soil Nitrogen as parts per million			
	Aerobic		Water-logged		Aerobic		Water-logged	
	NO_2-N	NH_3-N	NO_2-N	NH_3-N	NO_2-N	NH_3-N	NO_2-N	NH_3-N
0 . .	0·76	52·0	0·76	52·0	0·17	48·0	0·17	84·0
1 . .	2·41	51·0	Slight	37·7	0·67	61·0	0·19	37·5
2 . .	1·92	40·2	Do.	35·8	0·29	58·6	0·17	39·2
3 . .	1·20	40·9	Do.	37·2	0·63	61·0	0·13	..
4 . .	1·20	49·2	Do.	42·5	0·31	59·7	0·10	42·8
5 . .	2·38	45·8	Do.	42·6	0·60	58·2	0·10	44·6
6 . .	4·04	39·0	Do.	37·0	0·61	61·3	0·10	46·5
7 . .	3·33	62·0	Do.	56·9	0·66	56·3	0·46	50·0
8 . .	0·88	25·3	Do.	24·7	0·37	48·7	0·19	51·0
9 . .	0·52	28·2	0·74	45·5	0·11	49·8	0·23	52·2
10 . .	0·59	34·5	0·90	44·0	0·10	53·0	0·17	43·9
11 . .	..	31·1	..	42·2	..	57·0	..	64·3
12 . .	..	34·2	..	44·4	..	66·7	..	52·3

In spite of the fluctuations in the figures it is evident that during the first few days less nitrite and ammonia were formed in the soils kept under water than in those under ordinary arable conditions. A similar reduction of nitrate, although not so marked, has already been observed in the water-logged cultures of these soils receiving farmyard manure, sulphate of ammonia or no nitrogen.

In both the soils the diminution of nitrate nitrogen under water-logged condition was much in excess of the nitrite and ammonia formed. The intense activity of de-nitrifying organisms in the submerged soils is vividly brought out by the fact that not only a very large portion of the added nitrate was reduced within a few days but also the ammonia and nitrite already present in the soil suffered reduction to a marked extent.

It may be seen that after the first week the accumulation of ammonia in the water-logged cultures exceeded that in the corresponding aerobic cultures, the difference being more pronounced in *Dorsa* than in *Matasi* soil. Probably this is due to the efficiency of the de-nitrifying bacteria diminishing after a time owing to increase in numbers as has been observed by one of the authors in relation to the effect of bacterial population on the production of carbon dioxide from soil.

EXPERIMENT IN CULTURE SOLUTIONS

The following experiments were carried out for studying more effectively the comparative influence of the different treatments on the nitrate-reducing organisms in these soils :—

One hundred grm. portions of soils were treated individually with farmyard manure and sulphate of ammonia, each at the rate of 100 mg. of nitrogen per kg. of soil. These cultures along with those receiving no nitrogen were incubated for three weeks both under aerobic and water-logged conditions, and afterwards 100-c.c. lots of nutrient solutions in separate 250 c.c. Erlenmeyer flasks were inoculated with one grm. of soil (on dry basis) from each of the treatments. The nutrient solution was prepared by sterilizing separately and mixing equal quantities of the following :

(*a*) 2·5 grm. potassium nitrate per litre ;

(*b*) 2·0 grm. potassium phosphate, 0·4 grm. magnesium sulphate, 0·2 grm. each calcium chloride, potassium chloride and sodium chloride, and 0·004 grm. ferric chloride per litre.

The flasks were incubated at 32°C. and 2 c.c. of the solution withdrawn daily by sterilized pipettes for nitrate determination. The mean daily nitrate nitrogen for four-day intervals are given in Table IX.

TABLE IX

Nitrate-nitrogen in mg. per litre (means of four days)

Treatments		*Dorsa* soil				*Matasi* soil			
		Days			Mean difference in NO_3 (AE—WL)	Days			Mean difference in NO_3 (AE—WL)
		1-4	5-8	9-12		1-4	5-8	9-12	
		NO_3	NO_3	NO_3		NO_3	NO_3	NO_3	
No nitrogen	AE. .	323	300	323	+120	305	300	323	+20
	WL..	263	278	285		285	300	323	
Ammonium sulphate	AE. .	322	315	330	+143	293	338	341	+23
	WL..	240	292	292		285	345	319	
Farmyard manure	AE. .	293	330	313	+13	325	341	345	+118
	WL..	293	322	308		293	292	308	

AE = Aerobic.
WL = Water-logged.

The solution cultures inoculated with soils from the submerged treatments gave less nitrate than those inoculated with soils from the corresponding aerobic treatments. It is, therefore, evident that the de-nitrifying bacteria occurring in the water-logged soils functioned effectively after inoculation into solution cultures.

The mean difference in the amounts of nitrate in cultures inoculated, respectively, with soils incubated under aerobic and water-logged conditions forms for each treatment a measure of the activity of the nitrate-reducing organisms. Accordingly, it may be seen that in the presence of a mixed soil flora the de-nitrifying bacteria in the sulphate of ammonia and no nitrogen submerged treatments show greater vigour and activity in *Dorsa* than in *Matasi* soil; with farmyard manure, on the contrary, de-nitrification is much more intense in the latter so ilthan in the former. It is rather striking that the lowering of the bacterial numbers as effected by these treatments in water-logged soil cultures also leads to similar conclusions.

The above data further help in understanding the relative influence of sulphate of ammonia and farmyard manure (as compared with no nitrogen treatment) on the de-nitrifying bacteria present in either of the soils under water-logged conditions. Sulphate of ammonia, while proving beneficial to the de-nitrifiers in *Dorsa* soil, does not seem to exert any marked influence on these orgamisms in *Matasi* soil. Farmyard manure, however, has a distinctly toxic effect on the de-nitrifying bacteria in *Dorsa* soil although it favours very appreciably their activity in *Matasi* soil.

Carbon dioxide production

Some of the changes taking place in the soil as a result of anaerobic decomposition of green manure under water-logged condition have been studied by Harrison and Aiyer [1913-14]. Methane, hydrogen and carbon dioxide are generally evolved in the body of the soil; but at the surface the change is entirely different and the gases consist only of oxygen and nitrogen. The difference was attributed to a film of organisms which have the power of converting methane into carbon dioxide and this into oxygen. Söhngen [1906] first isolated from the soil *B. methanicus* which could transform methane partly into organic compounds and partly into carbon dioxide. Certain other common soil bacteria, capable of converting methane into carbon dioxide under anaerobic conditions have since been discovered. Münz [1915] has shown that some bacteria use the energy obtained by the oxidation of methane for chemeosynthetic utilization of carbon dioxide. This process may be represented by the equation $CH_4 + 2O_2 = CO_2 + 2\ H_2O +$ 218 Cals.

It was accordingly thought of interest to follow the evolution of free carbon dioxide under water-logged condition from the two rice soils, *Matasi* and *Dorsa*, each receiving farmyard manure, sulphate of ammonia and no nitrogen. For the sake of comparison the carbon dioxide produced from each of these soils under aerobic condition was also determined.

The total carbon dioxide produced in successive intervals of twenty-four hours was determined by aspirating air at the rate of four to five litres per day over 250 grms. of soil in 1000-c.c. Erlenmeyer flasks and absorbing it in a known volume of a standard baryta solution in Pettenkoffer tubes. The excess of baryta left in the tubes was determined by titration against standard hydrochloric acid. For each individual treatment two similar flasks were set up—one containing submerged soil and the other containing soil moistened to fifty per cent of its water-holding capacity. The doses of farmyard manure and sulphate of ammonia were each at the rate of 100 mg. nitrogen per kg. of soil.

The mean daily carbon dioxide production for four-day periods is given in Table X.

TABLE X

Carbon dioxide production (means of four days)

Treatments		CO_2 evolution. Daily rate in mg. per kg. of soil. Days			Total CO_2 produced in 12 or 13 days	Ratio of CO_2 evolution AE : WL
		1-4	5-8	9–12		
			(*a*) *Matasi soil*			
No nitrogen	AE. .	67·6	55·3	43·5	665·6	1·90
	WL..	24·2	25·8	37·6	350·2	
Ammonium sulphate.	AE. .	35·4	34·2	44·9	458·0	1·03
	WL..	60·0	28·1	23·6	446·7	
Farmyard manure.	AE. .	120·7	78·5	30·7	919·2	1·14
	WL..	86·4	92·5	23·6	809·7	
			(*b*) *Dorsa soil*			
No nitrogen	AE. .	97·9	69·3	61·0	912·7	1·36
	WL..	54·8	52·1	61·5	673·2	
Ammonium sulphate.	AE. .	82·9	38·1	39·8	643·2	1·83
	WL..	23·2	23·6	41·1	351·3	
Farmyard manure.	AE. .	75·0	73·5	20·3	675·2	1·48
	WL..	47·4	53·5	12·9	455·1	

AE=Aerobic.
WL=Water-logged.

It will be seen that in each case more carbon dioxide was evolved from the aerobic culture than from the corresponding water-logged one. From the ratios of carbon dioxide evolution between aerbic and submerged soils (given in Table X) it is, however, obvious that the production of carbon dioxide under water-logged condition did not lag much behind that under aerobic condition.

In the present set of experiments the surfaces of the submerged soils were carefully examined from time to time, but the formation of an algal film could not be traced in any of the cultures. Moreover, the presence of a film of micro-organisms on the surface would have almost completely prevented the evolution of free carbon dioxide by converting it into oxygen.

It has already been pointed out that the numbers of the strictly aerobic bacteria in the submerged cultures of both the soils, although much lower than those in the aerobic cultures, are sufficiently large to prove the presence of a fairly abundant supply of oxygen in the water-logged soils. It seems therefore reasonable to assume that as far as these soils are concerned the processes leading to the formation of carbon dioxide under submerged and aerobic conditions are more or less the same. The diminution in the production of carbon dioxide as a result of water-logging is according to expectation, since the supply of oxygen is less in the submerged culture than in the corresponding aerobic one.

The relative effect of water-logging on the carbon dioxide evolution is, however, not the same in the two soils. In the *Matasi* soil water-logging diminishes to a much greater extent the production of carbon dioxide in the untreated culture than in either of the treated cultures. The reverse is, however, the case in the *Dorsa* soil in which water-logging causes an appreciably greater diminution in the evolution of carbon dioxide in the treated cultures than in the control. Both under aerobic and submerged conditions more carbon dioxide was produced from *Dorsa* than from *Matasi*. With farmyard manure, on the contrary, more carbon dioxide was liberated from *Matasi* soil than from *Dorsa*. It is also evident that in the latter soil sulphate of ammonia inhibits carbon dioxide production to a very marked extent. It is interesting to point out here that the bacterial numbers, as obtained by plate counts, are higher in *Dorsa* than in *Matasi* soil. Also sulphate of ammonia distinctly lowers the bacterial numbers in *Matasi* soil.

Whatever the exact mechanism leading to the formation of carbon dioxide in water-logged soils, the results of the experiments summarised above tend to show clearly that there is a close similarity in the processes by which carbon dioxide is produced in these soils under aerobic and water-logged conditions.

Summary

The biochemical processes involved in the decomposition of farmyard manure and sulphate of ammonia under water-logged and aerobic conditions in two of the main rice soils of the Central Provinces were investigated in the laboratory.

The numbers of the strictly aerobic bacteria in the submerged cultures of both the soils, although much lower than those in the aerobic cultures, were sufficiently large to prove the presence of a fairly abundant supply of oxygen in the rice soils during the crop season.

Application of either farmyard manure or sulphate of ammonia depresses the population (both under aerobic and water-logged conditions) in the heavier soil, *Dorsa*, while no such effect was noticed in the lighter soil, *Matasi*.

In *Matasi* soil water-logging in general led to a diminution in ammonia. In *Dorsa* soil there was, as a result of water-logging, no change in ammonia in cultures receiving farmyard manure, but the one which contained sulphate of ammonia showed a significant increase.

An inverse relationship exists between the bacterial numbers and ammonia accumulation.

The accumulation of nitrite was more in the heavier soil than in the lighter one. Nitrate was almost entirely absent in the submerged cultures of either soil.

In one of the soils (*Dorsa*) sulphate of ammonia proved beneficial and farmyard manure toxic to the de-nitrifying bacteria. But in the other soil (*Matasi*) while sulphate of ammonia did not show any effect on these organisms, farmyard manure distinctly favoured their activity.

The amount of carbon dioxide produced from each of these soils was measured. The results indicate that the processes leading to the formation of carbon dioxide under submerged and aerobic conditions are essentially the same.

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F

STATISTICAL NOTES FOR AGRICULTURAL WORKERS

NO. 16—A COMPLEX EXPERIMENT ON RICE AT THE CHINSURAH FARM, BENGAL, 1933-34

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INTRODUCTION

A large number of agricultural experiments are designed to elucidate information only on simple and direct issues, as for example, the relative efficiency of different doses of nitrogenous manures, or the yielding capacities of certain selected strains of rice. It is however often desirable to study the effect of one factor in relation to a second. For example, the agriculturist may be interested to know the individual requirements of doses of manures, etc., for his different varieties of rice; for it is well known that all varieties do not respond equally to the same manurial or cultural treatment. For studying such interactions between two or more factors affecting yields of crops, the most suitable technique is the 'Complex Experiment'. In such experiments, the relevant factors are superimposed and laid out in one large but compact plot design, with suitable block divisions for convenience of agricultural operations and elimination of soil heterogeneity. It has been found that the complex experiment "by allowing every one of a set of differential treatments to be combined with every one of another set, gives to each set of comparisons the same precision as if the whole experiment had been devoted to it alone. In addition, the design provides information on the same level of precision upon any differential responses that

may exist among the different treatments ". Finally, the large amount of data available in a complex experiment allow, after elimination of soil differences and treatment effects, sufficient degrees of freedom for a precise estimate of the residual error.

Object of the experiment

A study of response of rice plants to cultural treatments is for many reasons a very important problem for Bengal. In fact, for many parts of this province, a manurial treatment of rice fields is not essential, and is also not likely to be of much economic significance. What is particularly needed is to increase yield by a careful selection of heavy-yielding varieties suitable for each district and situation, and a detailed study of the effect of cultural operations such as spacing, water requirements at various stages of plant growth, time of planting and seedling number per hole, etc., on the yield per acre. Of these, the water requirement plays a very important part at different stages of the plant life and determines to a large extent the final yield; but for want of proper facilities, this factor could not be included in the present test. Three other factors, namely, time of planting, spacing, and seedling number per hole were combined in one complex experiment, and their effects on three varieties of rice with different ripening habits have been studied in all possible combinations.

Method of experiment

With a view to examine these mutual interactions, a four-fold complex experiment was designed in the Statistical Laboratory, Calcutta, and was laid out in the Chinsurah Farm, Bengal, in 1933, and comprised the following four sets of factors :—

(I) Three different varieties of rice :—

(1) *Nehara* :—with early ripening habit.
(2) *Bhasamanik* :—with medium ripening habit.
(3) *Bhasakalma* :—with late ripening habit.

(II) Five dates of planting :—

(1) July 16.
(2) August 1.
(3) August 16.
(4) September 1.
(5) September 16.

(III) Three spacings :—

(1) Six inches.

(2) Nine inches.

(3) Twelve inches.

(IV) Three different numbers of seedlings per hole :—

(1) 1 plant per hole.

(2) 2 plants per hole.

(3) Local method (2 to 4 plants per hole).

Taking all the four variates, there were ($3\times5\times3\times3$) or 135 different treatments. Each treatment was replicated three times, giving a total of 405 plots. The total area under the experiment was three acres, and the area per unit plot was 9 ft.$\times$ 18 ft. (1/269 acre approximately).

The lay-out adopted is shown in the annexed diagram. The experiment was divided into three blocks, each of which contained one set of all treatment . The plots of the same sowing date within each block were grouped together, and the position occupied by the sowing date groups within the blocks were determined at random. This grouping together of plots of the same sowing date was adopted to facilitate cultural operations. For the same reason, the three varieties were also laid out in compact rows. The nine combinations of spacings and seedling numbers were then thrown at random within each combination of date of planting and variety as shown in the diagram. Actual yields are given in the appendix.

	Sept. 1	July 16	Aug. 1	Sept. 16	Aug. 16
Nehara	D	A	D	E	I
	F	F	H	B	A
	E	B	I	F	H
Bhasa-manik	G	G	E	C	D
	A	E	A	I	F
	I	I	F	G	G
Bhasa-kalma	B	C	G	D	C
	C	H	C	H	B
	H	D	B	A	E
Bhasa-kalma	F	A	E	B	G
	G	I	F	C	A
	E	G	A	F	H
Nehara	I	H	C	G	D
	C	E	B	I	F
	A	D	G	A	B
Bhasa-manik	D	F	H	D	E
	H	C	D	E	I
	B	B	I	H	C
Bhasa-kalma	I	F	I	I	I
	D	B	H	E	D
	A	E	D	G	F
Bhasa-manik	E	A	G	B	B
	F	H	B	F	A
	C	D	C	A	H
Nehara	G	G	E	D	C
	H	I	A	H	G
	B	C	F	C	E

	Aug. 1	Aug. 16	Sept. 1	Sept. 16	July 16
Bhasa-kalma	D	D	C	E	E
	I	G	A	G	I
	E	C	H	C	G
Bhasa-manik	B	E	F	F	H
	G	F	I	D	A
	A	I	D	I	F
Nehara	F	H	G	H	B
	C	A	B	A	D
	H	B	E	B	C
Bhasa-kalma	B	I	I	B	B
	A	E	G	F	D
	F	F	F	D	F
Nehara	I	D	C	C	G
	D	C	A	I	A
	G	G	D	E	H
Bhasa-manik	C	A	E	H	E
	H	H	B	A	I
	E	B	H	G	C
Nehara	E	E	H	G	F
	B	F	I	F	I
	A	I	F	D	E
Bhasa-manik	D	D	C	E	D
	I	G	G	B	G
	F	C	A	C	B
Bhasa-kalma	C	B	E	H	C
	G	A	B	A	A
	H	H	D	I	H

	Sept. 16	Aug. 16	Sept. 1	Aug. 1	July 16
Bhasa-kalma	E	C	E	I	A
	I	H	C	E	G
	F	A	F	F	C
Bhasa-manik	B	I	I	B	F
	H	D	A	H	I
	G	G	H	G	H
Nehara	D	E	G	A	D
	C	B	D	C	B
	A	F	B	D	E
Bhasaa kalma	B	F	A	C	H
	G	E	H	A	B
	H	I	B	D	E
Nehara	F	G	I	H	F
	I	C	E	G	G
	E	D	F	B	I
Bhasa-manik	D	B	D	E	D
	A	H	C	I	A
	C	A	G	F	C
Nehara	G	H	A	F	H
	H	A	H	I	A
	B	I	C	E	C
Bhasa-manik	F	F	F	A	E
	E	C	B	C	B
	B	E	E	D	G
Bhasa-kalma	D	B	G	G	D
	C	D	D	H	I
	A	G	I	B	F

Different numbers of seedling per hole .	Spacing 6″	9″	12″
(1) 1 Plant per hole	A	B	C
(2) 2 Plants per hole	D	E	F
(3) Local methods (2 to 4 plants per hole) .	G	H	I

Diagram showing plan of the experiment.

The harvest was gathered between 18th November and 21st December 1933. Besides the yield of grain and straw, the following characters were also observed :—

(1) Date of flowering in the different plots.
(2) Height of the plants in the different plots.
(3) Length of inflorescence in the different plots.
(4) Number of tillers in the different plots.

Rainfall

The weekly totals of rainfall in the Chinsurah Farm during the season together with the number of rainy days in the week are given in Table I. It will be seen that the rainfall was well-distributed over April and May ; the downpour however began to be excessive in June. The normal for the month is 10·56 in., whereas the actual rain was 17·65 in. Besides, 7·28 in. of rain in one day (24th June), flooded the entire farm and there was great difficulty in draining out the water. Normal rain continued throughout July, August and September. The result was that transplanting had to be done under very unfavourable circumstances ; and the standing water, which persisted to a depth of more than one foot, affected the varieties considerably, and the tillering was uniformly poor. The young seedlings thus did not get a good start, and the final yields were rather poor.

TABLE I

Weekly totals of rainfall in inches at Chinsurah Farm, 1933

Period	Total rain-fall	No. of rainy days	Period	Total rain-fall	No. of rainy days
March 1—31 .	0·00	0	July 8—14 .	1·35	5
			,, 15—21 .	3·12	5
April 1—7 .	0·00	0	,, 22—28 .	1·60	7
,, 8—14 .	2·38	3	,, 29—4 Aug.	1·34	4
,, 15—21 .	0·97	1			
,, 22—28 .	1·50	2	Aug. 5—11 .	2·68	6
,, 28—5 May	1·53	3	,, 12—18 .	4·80	7
			,, 19—25 .	6·03	7
May 6—12 .	0·88	4	,, 26—1 Sept.	1·77	5
,, 13—19 .	0·54	3			
,, 20—26 .	0·37	1	Sept. 2—8 .	2·07	4
,, 27—2 June	1·96	5	,, 9—15 .	0·39	3
			,, 16—22 .	6·98	5
June 3—9 .	2·38	2	,, 23—29 .	1·18	4
,, 10—16 .	1·81	5	,, 30—6 Oct.	1·54	1
,, 17—23 .	5·01	7			
,, 24—30 .	8·12	5	Oct. 7—13 .	0·00	0
			,, 14—20 .	2·56	5
			,, 21—27 .	0·67	2
July 1—7 .	4·25	6	,, 28—3 Nov.	0·00	0

STATISTICAL ANALYSIS OF DATA AND DISCUSSION OF RESULTS

The analysis of variance† of the yield of grain is shown in Table II. The actual yield data are given in the appendix.

TABLE II

Analysis of variance, yield of grain in tolas

Factor of variation	Degrees of freedom	Sum of squares	Variance	Mean variance / Error variance
Block	2	288,195	144,097·50	..
Date of planting	4	9,559,367	2,389,841·75	78·45**
Error	8	243,685	30,460·63	..
Variety	2	1,417,033	708,516·50	120·12**
Planting × Variety	8	236,867	29,608·37	5·01**
Error	20	117,966	5,898·30	..
Spacing	2	129,695	64,847·50	47·54**
Seedling	2	65,381	32,690·50	23·96**
Seedling × Spacing	4	5,583	1,395·75	1·02
Planting × Seedling	8	19,871	2,483·87	1·82
Planting × Spacing	8	42,563	5,320·37	3·90*
Varieties × Seedling	4	4,450	1,112·50	0·81
Varieties × Spacing	4	5,226	1,306·50	0·95
Planting × Variety × Seedling	16	63,854	3,990·87	2·92*
Planting × Variety × Spacing	16	47,239	2,952·43	2·16*
Planting × Seedling × Spacing	16	23,070	1,441·87	1·05
Variety × Seedling × Spacing	8	9,586	1,198·25	0·87
Planting × Variety × Seedling × Spacing	32	30,295	946·71	0·69
Error	239	326,007	1,364·05	..

N.B.—Items marked with two stars (**) are significant on a level of one per cent probability, and those marked with a single star (*) on the level of five per cent probability.

†The yield of one plot was found missing. This was reconstructed by the method of minimising the error variances described by Yates in "the Analysis of Replication Experiments when the Field Results are Incomplete", *Emp. J. Expt. Agric.*, *Vol.* 1, 1930, 129-142.

From Table II the significant effects can at once be located—

(I) Among primary effects—
  (1) the date of planting.
  (2) variety.
  (3) spacing, and
  (4) number of seedlings
  are all significant.

(II) First-order interactions between—
  (5) date of planting and variety, and
  (6) date of planting and spacing
  are significant.

(III) Second-order interactions between—
  (7) date of planting, variety, and number of seedling,
  (8) date of planting, variety, and spacing
  are significant.

(IV) The third-order interaction is inappreciable.

These results may now be shown quantitatively. The primary results are shown in Table III.

TABLE III

Mean yield of grain in tolas per plot

Varieties	Mean yield	Date of planting	Mean yield	Spacing	Mean yield	No. of seed-lings	Mean yield
Nehara	278.62	July 16	434.27	6 in.	374.03	1	337.25
Bhasamanik . . .	423.10	Aug. 1	477.99	9 in.	357.41	2	356.77
Bhasakalma	360.31	Aug. 16	438.75	12 in.	330.59	Local	368.00
		Sept. 1	363.25				
		Sept. 16	55.80				
Mean	354.01		354.01		354.01		354.01
Standard error . . .	2.20		4.22		3.17		3.17

Variety.—The medium ripening *Bhasamanik* is by far the largest yielder, then comes the late variety *Bhasakalma*, while the early *Nehara* gives the lowest yield. The differences are considerable in magnitude.

Date of planting.—The highest yield was obtained for the second date of planting (August 1); the first (July 16) and the third (August 16) are practically identical, while the fourth (September 1) is decidedly inferior to the first three, and the last one (September 16) is a clear failure.

Spacing.—The closest spacing (6 in.) gives the highest yield, nine in. spacing gives four per cent lower yield, and twelve in. spacing twelve per cent lower yield than the six in. spacing.

Number of seedlings per hole.—The local method (two to four seedlings) gives the best results ; yields with one or two seedlings are significantly lower.

Interactions.—In order to appreciate the full implications of the primary effects, an examination of the significant interactions is essential.

The interactions between " variety " and " date of planting " are shown in Table IV.

TABLE IV

Interaction of date of planting and variety (mean yield of grain in tolas per plot)

Variety	Date of planting					Mean
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
Nehara	372.81	396.90	357.29	260.35	5.74	278.62
Bhasamanik . . .	474.68	541.18	498.68	439.72	161.22	423.10
Bhasakalma	455.31	495.88	460.29	389.68	0.40	360.31
Mean	434.27	477.99	438.75	363.25	55.80	...
	Standard error = 4.93					
Difference between Varieties—						
Bhasamanik—Bhasakalma .	19.37	45.30	38.39	50.04	160.82	...
Bhasakalma—Nehara .	82.50	98.98	103.00	129.33	—5.34	...
Bhasamanik—Nehara .	101.87	144.28	141.39	179.37	155.48	...
	Standard error of difference = 6.97					

It will be seen that all the differences except one are statistically significant. Ignoring the 5th planting (September 16) for the present, since for both *Nehara* and *Bhasakalma*, it was a failure, it is evident that the 2nd (August 1), 3rd (August 16), and 4th dates (September 1) give much larger differences compared to the first (July 16).

The difference between *Bhasamanik* and *Bhasakalma* is thus less marked for July 16 than for August 1, August 16 or September 1. Similarly, the difference between *Bhasakalma* and *Nehara* is more marked for August 16 and September 1 than for July 16 or August 1. As regards *Bhasamanik* and *Nehara*, the difference is more marked for August 1 and August 16, and particularly for September 1 than for July 16.

The interactions between " date of planting " and " spacing " are given in Table V.

TABLE V

Interaction of date of planting and spacing (mean yield of grain in tolas per plot)

Spacing	Date of planting					Mean
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
(6 in.)	453.35	479.35	459.20	401.57	76.68	374.03
(9 in.)	437.61	486.48	438.74	371.83	52.39	357.41
(12 in.)	411.85	468.14	418.33	316.35	38.29	330.59
Mean	434.27	477.99	438.75	363.25	55.80	354.01
	Standard error = 7.17					
Differences—						
(6 in.)—(9 in.) . . .	15.74	—7.13	20.46	29.74	24.29	...
(9 in.)—(12 in.) . . .	25.76	18.34	20.41	55.48	14.10	...
(6 in.)—(12 in.) . . .	41.50	11.21	40.87	85.22	38.39	...
	Standard error of difference = 10.10					

A spacing of six in. is practically of no advantage over nine in. for the first two dates of planting. Its usefulness increases very rapidly as the date of planting is more and more delayed.

Between nine in. and twelve in., the difference was always significantly in favour of the nine in., except for the second date, when the difference was not appreciable. Here also a closer spacing is found to be more useful for the late planting.

Between six in. and twelve in., the differences are considerable, but still for the second date of planting the difference is insignificant.

We thus find that neglecting the 5th date of planting (September 16) which was a failure in certain cases :—

(1) A closer spacing is extremely useful if the date of planting is delayed.

(2) For the normal date of planting in this part of Bengal (August 1), the yields of grain for all the three spacings did not differ appreciably. The conclusion cannot however be considered final, since in this particular year the first date of planting was prejudicially affected by heavy rain.

(3) For the early planting (July 16), the difference between nine in. and twelve in., also between six in. and twelve in. is significant.

The second-order interactions between "variety", "date of planting", and "spacing" are given in Table VI, and the various differences in Tables VII and VIII.

TABLE VI

Interaction of variety, planting and spacing (mean yield of grain in tolas per plot)

Variety	Spacing	Date of planting					Mean
		July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
Nehara	6 in.	386.8	396.0	387.5	298.8	7.8	295.38
	9 in.	376.1	406.2	347.3	280.4	5.7	283.14
	12 in.	355.6	388.6	337.1	201.8	3.8	257.22
Bhasamanik	6 in.	497.0	549.1	513.4	469.2	221.9	450.12
	9 in.	480.8	541.0	500.6	436.8	151.4	422.12
	12 in.	446.4	533.4	482.0	413.1	110.4	397.06
Bhasakalma	6 in.	476.4	492.9	476.6	436.7	0.4	376.60
	9 in.	455.9	512.3	468.3	398.2	0.1	366.96
	12 in.	433.6	482.4	436.0	334.2	0.7	337.38
Mean	...	434.27	477.99	438.75	363.25	55.80	354.01

Standard error = 12.31

Failure of *Nehara* and *Bhasakalma* on September 16 is evident from the last column.

TABLE VII

Mean difference in yield (in tolas per plot) between 6-in. and 9-in. spacing

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara	10.7	—10.2	40.2	18.4	2.1
Bhasamanik	16.2	8.1	12.8	32.4	70.5
Bhasakalma	20.5	—19.4	8.3	38.5	0.3
Mean	15.80	—7.17	20.44	29.77	24.30

Standard error of difference = 17.35

Table VII shows that differences between six in. and nine in. are insignificant for July 16 and August 1 for all the varieties.

In Table V the superiority of six in. over nine in. was significant for August 16; this because *Nehara* (early variety) alone showed this tendency while for the two other varieties the differences were insignificant.

For September 1, both *Bhasamanik* and *Bhasakalma* gave better yields for six in. spacing than for nine in. For September 16, *Bhasamanik* alone gave an appreciable yield which certainly shows its remarkable adaptability.

TABLE VIII

Mean difference in yield (in tolas per plot) between 9-in. and 12-in. spacing

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara	20.5	17.6	10.2	78.6	1.9
Bhasamanik . . .	34.4	7.6	18.6	23.7	41.0
Bhasakalma . . .	22.3	29.9	32.3	64.0	—0.6
Mean	25.73	18.33	20.36	55.43	14.10

Standard error of difference = 17.35

Table VIII indicates for July 16 a big difference for *Bhasamanik*, while the two others show no appreciable difference as the spacing is changed from nine in. to twelve in. This significant increase of *Bhasamanik* is responsible for the positive response of July 16 in the first-order interaction.

For August 16, the difference for *Bhasakalma* alone is on the verge of significance. For September 1 both *Nehara* and *Bhasakalma* show considerable increase, while *Bhasamanik* gives practically the same yield for both spacings.

For September 16, *Nehara* and *Bhasakalma* figures are unreliable; but *Bhasamanik* shows a definite increase in yield for nine in. spacing against twelve in.

Table IX gives the second-order interactions between "date of planting", "variety", and "seedlings per hole", actual differences being given in Tables X and XI.

TABLE IX

Interaction of planting, variety and seedlings

Variety	Number of seedlings per hole	Date of planting					Mean
		July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
Nehara	1	381.4	378.5	343.6	228.0	3.8	267.06
	2	355.6	430.2	358.4	255.7	6.4	281.26
	Local method	381.4	382.0	369.8	297.4	7.1	287.54
Bhasamanik	1	463.6	538.1	489.3	414.6	102.4	401.60
	2	479.7	539.8	497.3	440.7	171.5	425.80
	Local method	480.8	545.7	509.5	463.8	209.8	441.92
Bhasakalma	1	436.2	487.2	418.6	372.9	0.6	343.10
	2	469.1	487.6	477.6	381.8	0.3	363.28
	Local method	460.6	512.8	484.7	414.3	0.3	374.54
Mean	...	434.27	477.99	438.75	363.25	55.80	354.01

Standard error = 12.31

TABLE X

Mean difference in yield (in tolas per plot) between one and two seedlings per hole

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara	—25.8	51.7	14.8	27.7	2.6
Bhasamanik	16.1	1.7	8.0	26.1	69.1
Bhasakalma	32.9	0.4	59.0	8.9	—0.3

Standard error = 17.35

For July 16, all the differences are insignificant. For August 1, *Nehara* alone gives significant increase for two seedlings. For August 16, the differences for *Nehara* and *Bhasamanik* are not significant ; but it is significant for *Bhasakalma*. It may be noted that *Nehara* gives a maximum yield at this date.

For September 1, all the differences are insignificant. For September 16, *Bhasamanik* shows a definite increase for two seedlings over one seedling per hole.

TABLE XI

Mean difference in yield (in tolas per plot) between two seedlings per hole and local method

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara . .	25·8	—48·2	11·4	41·7	0·7
Bhasamanik .	1·1	5·9	12·2	23·1	38·3
Bhasakalma .	– 8·5	25·2	7·1	32·5	0·0

Standard error = 17·35

For all dates of planting the ' local method ' is as good as the two-seedling method for both *Bhasakalma* and *Bhasamanik* ; the latter however shows an increased yield under the ' local method ' only for September 16.

Nehara has given an anomalous result. For July 16, August 16, and September 16, the differences are insignificant ; for September 1, the difference is significantly in favour of ' local method ', while for August 1, the two-seedling method has given an appreciably higher yield than the local one. This particular behaviour for August 1 requires further investigation, before it can be considered as established.

All other interactions are insignificant.

It must be added that the results discussed above were almost certainly affected by the conditions of the season, and it is not unlikely that at least some of them will be modified under varying seasonal conditions. With a view to obtain information on this seasonal influence, the experiment is being repeated for three consecutive years, and the results will be discussed in subsequent notes.

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Summary

The present paper gives the results of a four-fold complex experiment on rice with three varieties with different ripening habits (*Nehara*, *Bhasamanik*, *Bhasakalma*) ; five dates of planting (July 16, August 1 and 16, September 1 and 16); three spacings (6-in., 9-in., 12-in). ; and three different numbers of seedlings per hole (one plant, two plants, and local method) laid out in orthogonal and randomized plots in three blocks.

(1) All the three varieties of rice give the largest yield when planted on August 1. For this date of planting, the three different spacings, (6-in.), (9-in.) and (12-in.), make no appreciable difference in the total yield, although the total number of plants per plot in the three cases is in the proportion of 6 : 4 : 3. Again, the yield is not significantly different whether the method of planting is of 'local type' or one seedling, or two seedlings per hole, except for *Nehara* (early ripening) which shows the highest yield for two seedlings per hole.

(2) The system of spacing and seedling-number per hole becomes increasingly important as the date of planting is delayed. Thus a closer spacing as well as a larger number of seedlings are necessary for later dates of planting. This is to be expected from physiological considerations of plant growth, but the quantitative results obtained in the present experiment have indicated the remarkable adaptability of *Bhasamanik* (medium ripening) for all the dates of planting and spacings. Even for the latest date of planting (September 16) it gave fairly large yield with all spacings and seedling numbers. *Bhasakalma* (late) and particularly *Nehara* (early) lack this important quality, and need closer spacing and greater seedling number for maintaining a high yield. Both of them failed for September 16 under all conditions of spacing and seedling number.

Appendix

YIELDS OF RICE

Field 1

	September 1		July 16		August 1		September 16		August 16	
Nehara	D	264·0	A	376·0	D	381·5	E	10·0	I	308·5
	F	211·0	F	329·0	H	337·5	B	3·0	A	316·5
	E	296·0	B	338·0	I	344·0	F	2·0	H	322·5
Bhasa-manik	G	450·0	G	405·0	E	451·0	C	35·0	D	415·5
	A	416·0	E	418·5	A	475·0	I	141·0	F	400·5
	I	433·0	I	394·5	F	460·5	G	314·0	G	419·0
Bhasa-kalma	B	367·5	C	393·0	G	421·0	D	0·5	C	357·5
	C	243·0	H	406·0	C	397·5	H	*nil*	B	411·0
	H	398·5	D	406·0	B	423·5	A	*nil*	E	430·5
Bhasa-kalma	F	318·5	A	388·5	E	412·5	B	*nil*	G	451·5
	G	450·5	I	365·5	F	405·5	C	1·0	A	409·5
	E	374·0	G	401·5	A	404·5	F	*nil*	H	398·5
Nehara	I	207·0	H	333·0	C	301·5	G	16·5	D	313·0
	C	163·5	E	308·0	B	344·5	I	2·5	F	268·0
	A	225·5	D	306·5	G	340·0	A	8·0	B	298·5
Bhasa-manik	D	413·5	F	339·0	H	466·5	D	231·0	E	404·5
	H	439·0	C	370·5	D	448·5	E	173·0	I	403·5
	B	389·0	B	393·0	I	444·0	H	229·0	E	370·0
Bhasa-kalma	I	373·0	F	355·0	I	420·0	I	1.5	I	382·5
	D	425·5	B	377·0	H	455·5	E	*nil*	D	440·5
	A	413·0	E	372·5	D	380·5	G	*nil*	F	375·5
Bhasa-manik	E	457·0	A	382·0	G	464·0	B	120·5	B	452·0
	F	394·0	H	405·0	B	479·5	F	127·0	A	482·0
	C	351·0	D	448·0	C	462·5	A	174·0	H	462·0
Nehara	G	380·5	G	376·0	E	420·0	D	8·5	C	326·0
	H	381·5	I	327·0	A	415·5	H	5·0	G	413·0
	B	318·5	C	355·5	F	431·0	C	3·0	E	350·0

Field 2

	August 1		August 16		September 1		September 16		July 16	
Bhasa-kalma	D	573·0	D	612·5	C	375·0	E	1·0	E	551·5
	I	556·0	G	595·5	A	499·0	G	1·5	I	465·0
	E	585·5	C	509·0	H	496·0	C	3·0	G	519·0
Bhasa-manik	B	580·0	E	558·0	F	468·0	F	230·0	H	550·0
	G	577·0	F	570·0	I	492·0	D	416·0	A	542·0
	A	598·5	I	553·5	D	550·0	I	174·0	F	523·0
Nehara	F	417·0	H	375·5	G	295·0	H	4·0	B	417·0
	C	363·0	A	405·5	B	222·0	A	4·0	D	375·0
	H	418·5	B	292·5	E	278·0	B	5·0	C	352·0
Bhasa-kalma	B	518·0	I	523·0	I	333·5	B	*nil*	B	437·0
	A	579·0	E	576·5	G	488·0	F	1·0	D	516·5
	F	512·0	F	501·0	F	292·0	D	*nil*	F	406·5
Nehara	I	422·5	D	464·0	C	171·0	C	1·0	G	393·5
	D	460·5	C	364·5	A	337·5	I	4·0	A	378·5
	G	394·5	G	417·0	D	294·0	E	5·0	H	391·0
Bhasa-manik	C	549·0	A	586·5	E	410·0	H	198·0	E	473·5
	H	622·0	H	572·0	B	444·5	A	205·0	I	455·5
	E	594·0	B	529·0	H	506·0	G	258·5	C	395·0
Nehara	E	452·0	E	386·0	H	297·0	G	2·5	F	331·5
	B	405·0	F	345·0	I	312·5	F	4·0	I	325·0
	A	367·0	I	347·0	F	218·0	D	9·0	E	353·0
Bhasa-manik	D	602·0	D	548·0	C	399·0	E	182·0	D	451·0
	I	600·0	G	534·0	G	479·5	B	153·5	G	483·0
	F	571·0	C	507·0	A	469·0	C	100·0	B	416·0
Bhasa-kalma	C	540·0	B	503·5	E	349·5	H	*nil*	C	384·0
	G	548·0	A	312·5	B	360·5	A	1·5	A	445·0
	H	594·0	H	573·5	D	471·0	I	*nil*	H	477·0

Field 3

	September 16		August 16		September 1		August 1		July 16	
Bhasa-kalma	E	*nil*	C	394·0	E	462·0	I	580·5	A	547·0
	I	*nil*	H	486·0	C	355·0	E	551·0	G	518·0
	F	*nil*	A	517·0	F	361·0	F	481·5	C	456·0
Bhasa-manik	B	118·0	I	598·0	I	460·0	B	549·5	F	512·0
	H	191·0	D	616·0	A	501·0	H	554·5	I	527·0
	G	211·5	G	525·5	H	429·0	G	608·5	H	484·0
Nehara	D	3·5	E	386·0	G	274·0	A	429·0	D	408·0
	C	2·0	B	329·5	D	302·0	C	372·5	F	391·0
	A	3·0	F	330·5	B	176·0	D	430·0	E	408·5
Bhasa-kalma	B	*nil*	F	422·5	A	364·5	C	449·0	H	496·
	G	*nil*	E	487·5	H	397·0	A	521·0	B	499·0
	H	*nil*	I	458·0	B	379·0	D	486·0	E	487·0
Nehara	F	10·0	G	392·0	I	247·0	H	427·5	F	381·0
	I	5·5	C	376·5	E	273·0	G	346·0	G	434·5
	E	5·5	D	383·5	F	165·5	B	409·0	I	407·5
Bhasa-manik	D	173·5	B	502·0	D	458·0	E	572·0	D	561·0
	A	13·5	H	518·0	C	375·0	I	575·0	A	577·0
	C	2·0	A	494·5	G	486·0	F	573·0	C	501·0
Nehara	G	15·0	H	385·0	A	317·0	F	438·0	H	445·5
	H	8·5	A	383·0	H	282·0	I	407·5	A	433·0
	B	5·0	I	368·0	C	120·5	E	441·5	C	391·5
Bhasa manik	F	8·5	F	455·0	F	346·0	A	582·5	E	591·0
	E	2·5	C	480·5	B	387·0	C	566·0	B	596·0
	I	176·0	E	508·0	E	470·0	D	586·0	G	623·0
Bhasa-kalma	D	*nil*	B	353·0	G	436·0	G	523·5	D	546·0
	C	*nil*	D	452·0	D	383·0	H	517·0	I	497·0
	A	*nil*	G	499·0	I	356·5	B	552·5	F	580·5

INFLUENCE OF STORAGE ON SOME OF THE FIBRE-PROPERTIES OF DHARWAR COTTON

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(With two text-figures)

I. Introduction

In the testing of cottons for the determination of fibre-properties, it frequently happens that due to pressure of work some samples remain untested for a certain period of time. The question arises whether any change in the fibre-properties is caused by storage, if clean samples are kept for about a year under exceptionally favourable condition, as regards humidity and protection from rain, etc. The object of the present investigation is to find out whether and to what extent the more common of the fibre-properties exhibit any variation due to storage under these special conditions.

Important and extensive investigations have already been done by Ahmad [1935] on the effect of storage of *kapas* on the quality of cotton. A study of the deterioration of cotton stacked in open plinths at Karachi has also been made by the same author [Ahmad, 1934]. The work reported in this paper was undertaken in 1929 and tests on the three principal fibre-properties, length, weight and strength were carried out at an interval of six months for a period of three and a half years.

II. Experimental

(*a*) *Material*

The cotton selected for the experiments was Dharwar I as it was found by Turner [1929] to show very little variation in fibre-properties in different seasons. The test sample (season 1928-29) was kindly supplied by the Cotton Breeder, Dharwar.

(*b*) *Storage*

A sample of about 2 lbs. was preserved in a loose condition in a cloth bag, which was kept in a clean and dry cupboard. Samples were taken from this bag periodically for testing. The first sample was taken after six months and the seventh (last) in September 1932, after a period of three and a half years.

(*c*) *Sampling*

In order to get a fully representative and satisfactory sample, sampling methods described by previous writers [Calvert and Summers, 1925; Turner,

1928 ; and Clegg, 1932] were followed. Each test sample was divided into two equal parts and each sub-sample was again divided into two equal parts, and this process was repeated until there were thirty-two parts in all. From each one of these groups, small bunches of fibres were drawn and cleaned one by one. These were drawn by hand and mixed together into a sliver. The hand sliver after drawing, mixing and doubling together was passed through a draw-box several times till a representative sliver, having most of the fibres parallel and straight, was obtained.

(*d*) *Experimental procedure.*

(*i*) *Mean fibre-length.*—The mean fibre-length was determined by two instruments, (1) Balls [1916] Sorter, and (2) Baer Sorter, using the methods suggested by Ahmad [1933, 1]. The results obtained by these two instruments serve as checks upon each other, though the Balls Sorter is really the more useful instrument. Two tests were made with the Balls Sorter and four with the Baer Sorter and the mean was calculated from the results.

(*ii*) *Fibre-weight per unit length.*—In determining the fibre-weight per inch, method prescribed by Ahmad [1933, 2] was followed. Fibres were cut into one-centimetre lengths by a suitable cutter and of these about 250 fibres were counted and weighed each time on a sensitive quartz micro-balance of the type described by Morton [1926]. In all about 3,000 fibres in groups of 250 fibres per lot were weighed. From these results the mean fibre-weight per inch was calculated. As cotton is hygroscopic, the values obtained for fibre-weight depend upon the moisture content at the time of weighing and this in turn depends upon the prevailing temperature and humidity of the atmosphere. In order to have comparable values, therefore, they were all corrected to 70 per cent relative humidity using the correction factor suggested by Urquhart and Williams [1926].

(*iii*) *Fibre-strength.*—Fibre-strength was determined by the O'neill's Hair Tester as modified by Mann and Pierce [1926]. About 300 fibres were tested singly on one-centimeter lengths and from the results obtained, the mean fibre-strength was calculated.

As fibre-strength is also much affected by the changes in the atmospheric humidity, the results were corrected by using the factor recommended by Ahmad [1933, 3]. The fibre-strength per unit fibre-weight per inch was calculated from the results obtained for mean fibre-strength (ozs.) and the mean fibre-weight per inch (10^{-6} ozs.).

(*iv*) *Colour.*—During each test, the lint was examined for feel and colour. These are rather difficult tests to make, for no numerical expression can be given to them. The colour was at first creamy, but as the period of storage increased, it became more and more dull, until finally there was a marked change in its appearance.

Discussion of results

The results obtained from these experiments are given in Tables I to VI, and are graphically represented in Figs. 1 and 2.

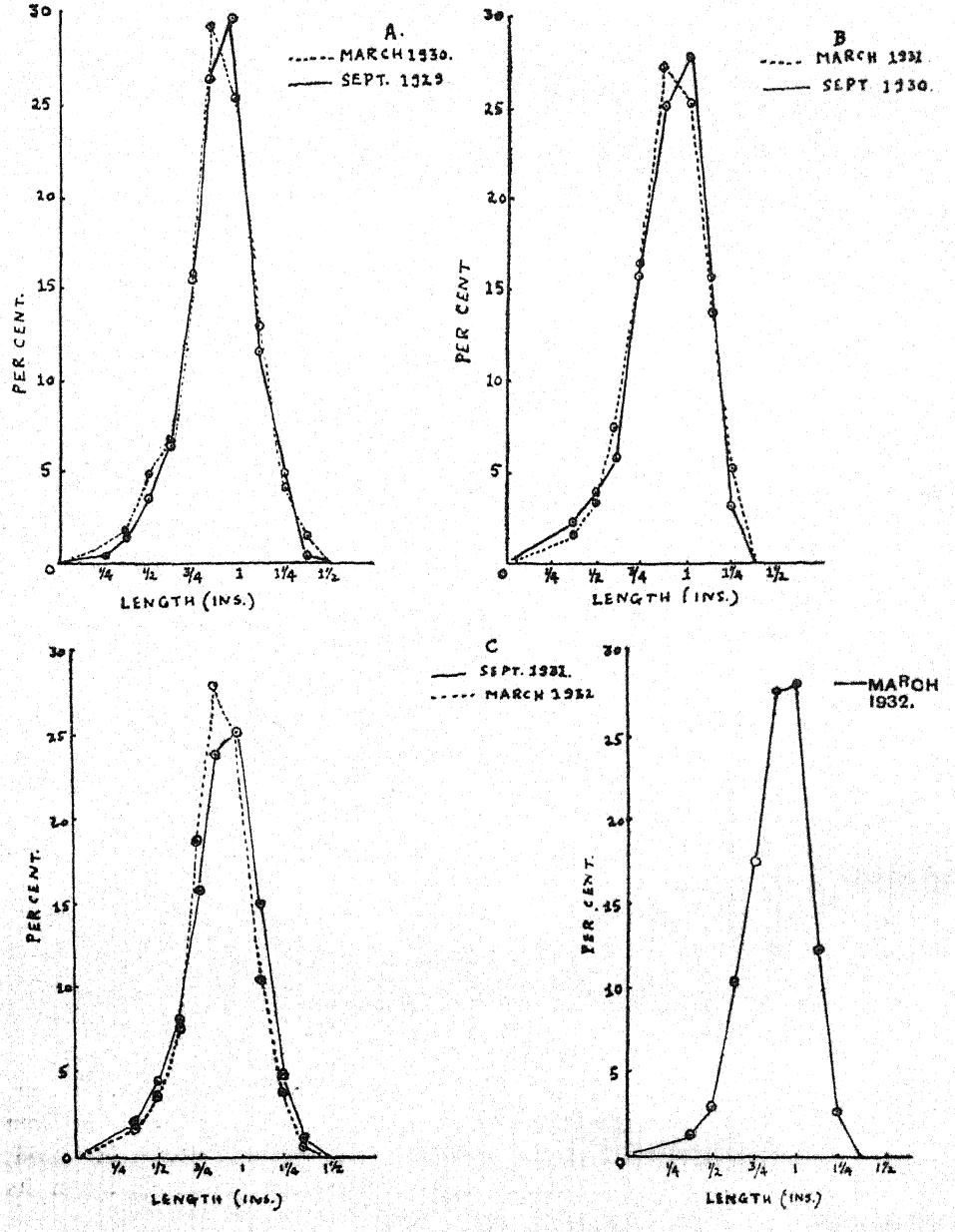

Fig. 1. Sorter diagram.

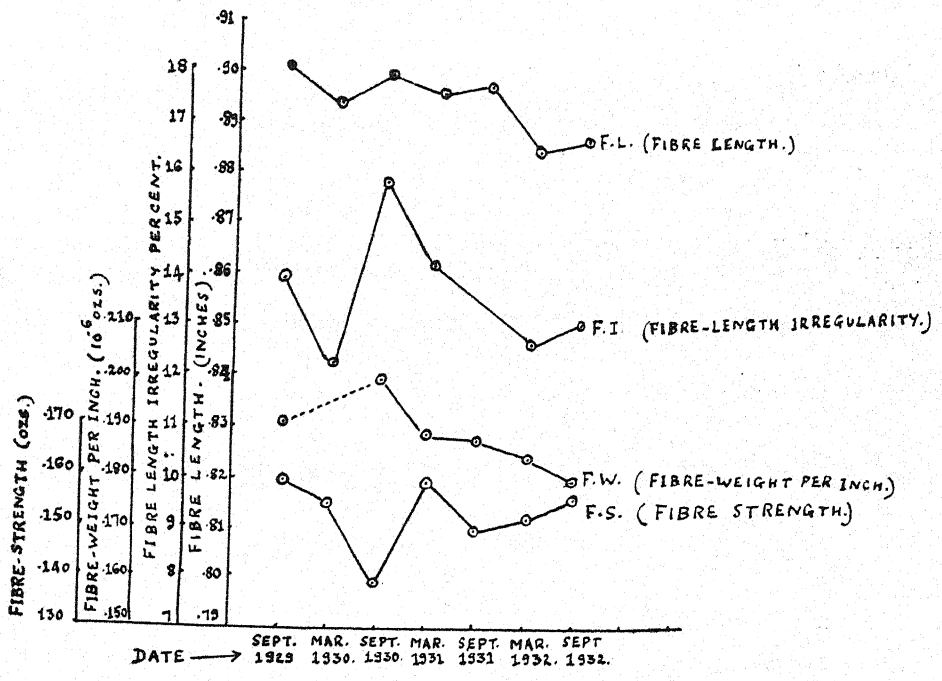

Fig. 2. Change of fibre-properties with date.

Fibre-length

Table I gives the mean fibre-length and fibre-length distribution.

TABLE I

Mean fibre-length and fibre-length distribution of Dharwar I (1928-29) cotton, when tested at six-monthly periods for three and a half years.

Mean group-length in eighths of an inch	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept 1931	March 1932	Sept. 1932
2	0·4	..	..	..	..	..	..
3	1·3	1·6	2·0	1·7	2·0	1·7	1·3
4	3·0	4·9	3·7	3·3	4·3	3·5	2·9
5	6·2	6·4	6·3	7·7	7·9	7·8	10·2
6	15·6	15·7	16·7	15·9	15·7	18·5	17·5
7	26·6	29·2	25·1	27·1	23·7	28·0	27·7
8	29·7	25·6	27·3	25·3	25·2	25·2	28·0
9	11·8	13·1	15·7	13·8	15·1	10·5	12·4
10	5·0	4·1	3·2	5·2	4·8	3·7	3·0
11	0·4	1·4	..	..	1·3	0·8	..
Mean fibre-length (inch) (Balls Sorter).	0·910	0·905	0·900	0·900	0·904	0·887	0·895
Mean fibre-length (inch) (Baer Sorter).	0·892	0·888	0·900	0·888	0·893	0·885	0·883
Mean of Balls and Baer tests.	0·901	0·896	0·900	0·894	0·898	0·886	0·889

From the data recorded in the above table it will be noted that the Ball Sorter readings show slight variation, the highest value obtained being in September 1929 and the lowest in March 1932. The percentage difference between the highest

value and the lowest, however, is only 2 5 and this cannot be considered as significant. The Baer Sorter results show less variation, the highest being 0·900 in September 1930 and the lowest in September 1932, the percentage difference being only 1·9 which again is non-significant. It may be stated that due to errors of sampling, preparation of slivers and some inherent defects in the instruments themselves, a difference in fibre-length up to 0·04 in. may easily be obtained. These differences cannot therefore be attributed to the effect of storage. The fibre-lengths on the different dates have been graphically represented in Fig. 2 along with other fibre-properties mentioned in this paper. It will be observed that practically no variation in fibre-length occurs throughout the period of storage. In Fig. 1, the fibre-length distribution is graphically represented.

From the data of Table I, the median and mode were also calculated and the values are given in Table II.

TABLE II

Median and mode of fibre-length of Dharwar I (1928-29) cotton, when tested at different periods

Test	Mean	Median	Mode
	Inch	Inch	Inch
September 1929	0·910	0·923	0·949
March 1930	0·905	0·904	0·903
September 1930	0·900	0·919	0·959
March 1931	0·900	0·919	0·933
September 1931	0·904	0·919	0·949
March 1932	0·887	0·895	0·911
September 1932	0·895	0·908	0·934

It will be noted from Table I, that the mode is in the group-lengths 8/8ths of an inch in four out of the seven cases and the rest in 7/8 ths groups. It will be further observed from Table II that the values for mean, median and mode are slightly higher in September than in March, possibly due to the effects of humidity and temperature. The former month is characterised at Dharwar by high humidity and low temperature and this is reversed in the month of March.

Fibre-length irregularity

From the Balls-Sorter results for fibre-length distribution, the fibre-length irregularity percentage was calculated using the equation given by Ahmad [1933, 4], *viz.*,—

$$\text{Mode—Mean} = 3\ (\text{Median—Mean}),$$

$$I = \frac{100 \times S}{W},$$

where S is the weight of fibres shorter than three-fourths of the mode, W the weight of representative sample and I is the fibre-length irregularity. The results are given in Table III.

TABLE III

Values of fibre-length irregularity of Dharwar I (1928-29) cotton, obtained during different periods

	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
Fibre-length irregularity in per cent	13·9	12·3	15·9	14·3	17·1	12·7	13·2

It will be noted from the data recorded in Table III, that the values have neither increased nor decreased continuously as a result of storage and that the September values are, as before, higher than those of March.

Table IV gives the values of mean fibre-weight per inch for the various tests.

TABLE IV

Mean fibre-weight values of Dharwar I (1928-29) cotton

	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
Mean fibre-weight per inch ($\times 10^{-6}$ ozs.)	0·191	..	0·200	0·189	0·188	0·185	0·180

It will be seen from the results given above, that the fibre-weight per inch shows some variation. The fibre-weight decreases year by year, probably due to storage. The difference between the highest value obtained for September 1930 and the lowest value obtained for September 1932 is about 10·5 per cent. It has to be emphasized that the difference between successive values is not significant and some of the differences may have been also due to sampling errors. The change of fibre-weight values with date has been graphically represented in Fig. 2.

Fibre-strength

Table V gives the values of the fibre-strength and fibre-strength per unit fibre-weight per inch of Dharwar I (1928-29) cotton when tested from time to time.

TABLE V

	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
Mean fibre-strength (ozs.).	0·158	0·155	0·138	0·158	0·150	0·153	0·157
$\frac{\text{Fibre-strength}}{\text{Fibre-weight}}$	0·83	..	0·69	0·84	0·80	0·83	0·87

From the results given in Table V, it appears that there is no change in fibre-strength as the period of storage is increased; the small difference between the highest value and the lowest is within the sampling errors. The fibre-strength per unit fibre-weight per inch is also practically constant excepting for September 1930 which is due to low fibre-strength recorded for that month.

Table VI gives the values of the frequency distribution for strength tests.

TABLE VI

Variation in the frequency distribution of fibre-strength tests of Dharwar I (1928-29) cotton, tested at different periods

Mean fibre-strength (grms.)	Frequency						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
0—0·5 . .	2	3	13	5	6	7	10
0·6—1·5 . .	6	9	29	20	28	30	32
1·6—2·5 . .	21	24	37	31	46	41	24
2·6—3·5 . .	30	35	66	45	48	60	27
3·6—4·5 . .	25	28	58	51	48	72	51
4·6—5·5 . .	30	34	53	44	39	50	48
5·6—6·5 . .	17	20	30	26	38	26	40
6·6—7·5 . .	14	10	11	15	20	11	33
7·6—8·5 . .	6	2	5	10	12	6	15
8·6—9·5 . .	3	..	2	3	7	1	10
9·6—10·5 . .	1	..	..	1	1	..	2
10·6—11·5 . .	..	..	..	1	1	..	..
Mean fibre-strength (grms.)	4·40	3·99	3·70	4·20	4·19	3·72	4·55
Relative humidity per cent	66	52	60	56	63	42	75
Mean fibre-strength at 70 per cent relative humidity (grms.)	4·48	4·38	3·90	4·50	4·23	4·33	4·44
Mean fibre-strength at 70 per cent relative humidity (ozs.)	0·158	0·155	0·138	0·159	0·150	0·153	0·157
Number of fibres tested.	155	165	304	254	297	305	292

The results of the tests conducted for a period of three and a half years indicate that there is practically no change in any of the fibre-properties determined. The results apply to Dharwar I type of cotton and the present sample was stored in exceptionally favourable conditions which have very little parallelism with ordinary conditions of storage of bales in godowns and therefore the results of these conclusions cannot be held as being valid for bales stored in the ordinary fashion. As the more common fibre-properties of this cotton do not exhibit appreciable change, it is permissible to make fibre tests on stored samples of this cotton, but it has to be emphasized that these should be kept under as dry conditions as possible, for moisture induces many micro-organisms such as fungus and bacteria to attack the fibres, in which case stored cotton may suffer much damage. The effect of storage on cotton by the attack of fungus and bacteria has not been investigated in the present experiments. It may be noted, that the fact that none of the fibre-properties indicated any significant change does not necessarily imply no deterioration, for due to bacterial and fungoid infection some of the cellulose may be eaten up, thus introducing more regions of weakness, which may not be ordinarily detected by the fibre-strength test due to the weakest link already present.

Summary

The experiments described in this paper refer to the controlled conditions of storage in laboratory, where most of the harmful effects of micro-organisms and climate are eliminated. A small sample (2 lb.) of Dharwar I (1928-29) cotton was stored in a cupboard in March 1929 for a period of three and a half years, and periodic tests were made at an interval of six months commencing from September 1929. There was found to be no significant change in any of the three fibre-properties,—length, weight and strength—studied. The colour of the cotton, however, was observed to change slightly. The practical conclusion that can be drawn from these experiments is that if due to pressure of work a sample of this cotton is left untested for a year or so, it is permissable to test it afterwards for these three fibre-properties, provided due care has been taken in storing the sample.

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VARIATION IN THE FIBRE-PROPERTIES OF SIMILAR STRAINS OF COTTON GROWN IN DIFFERENT TRACTS OF GUJARAT

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(With one text-figure)

I. Introduction

The Gujarat cotton belt comprises three distinct tracts, Broach, Surat and Viramgam, each place being the nucleus of a tract. Each tract produces a typical variety of cotton. As we proceed northwards from Surat the quality falls off. An examination of the maturity of Goghari E_2 during 1931-32 showed significant difference between Broach and Surat. Similarly 1027 A. L. F. differed between Surat and Viramgam. This diversity of results for a pure type naturally gave rise to the question, whether any general rule can be found whereby it will be possible to predict how a type is likely to behave when transferred from one tract to another, under normal conditions. This question is also important in hybridisation work, since a good type evolved in one tract may be taken up for crossing in another tract. A knowledge of the probable behaviour of any variety in different tracts is thus desirable.

II. Material

With this object in view lists of all the types grown on the three farms during 1931-32 were obtained and the following types which were the only common ones, chosen for the present investigation :—(1) 1027 A. L. F., (2) Broach Deshi 8, (3) Wagad 8×1027 A. L. F.—segregates 11-2, (4) 11-4 and (5) 12-1. These represent to a great degree the three tracts—1027 the standard for Surat, B. D.-8

specially selected at Broach, and W. 8×1027 specially hybridised for the Viramgam tract For this purpose representative* samples of 4-5 ozs. of each of these types, from the general lot, were obtained from the respective cotton breeders.

III. Methods

Tests on each sample were made to ascertain the fibre-length, fibre-weight per inch, and the maturity percentage. Length is the average of two Balls-Sorter tests. Fibre weight is an average of about 2,000 whole fibres counted and weighed in bunches of 100 by the method described by Ahmad [1933, 1]. The maturity percentage is determined by a slight modification of the Clegg [1932] method; while Clegg takes fibres from different sections of a Baer-Sorter pattern according to their length, here a bunch of fibres having approximately 250 fibres is drawn from the sliver and irrigated with eighteen per cent caustic soda solution after placing them between a micro-slide and a cover glass. A minimum of 500 fibres are tested for each sample. The rest of the experiment is similar to Clegg's.

IV. Discussion of results

1. Fibre-length

Table I below gives the average values of fibre-length in inches for all the samples tested. The percentage differences in length between the several tracts are also given.

Table I

Mean length and percentage differences in mean length in inches

No.	Cotton	Surat S	Broach B	Viramgam V	S—B	$\frac{S-B}{S}\times\frac{100}{1}$	V—B	$\frac{V-B}{V}\times\frac{100}{1}$	V—S	$\frac{V-S}{V}\times\frac{100}{1}$
1	1027 A. L. F. .	0·95	0·93	0·94	0·02	2·1	0·01	1·0	—0·01	—1·1
2	B. D.-8 . . .	0·93	0·89	0·87	0·04	4·3	—0·02	—2·3	—0·06	—6·9
3	W.-8×1027 11-4 .	0·87	0·94	0·87	—0·07	—8·1	—0·07	—8·0	0·00	0·0
4	W.-8×1027 11-2 .	0·90	0·89	0·90	0·01	1·1	0·01	1·1	0·00	0·0
5	W.-8×1027 12-1 .	0·89	0·90	0·89	—0·01	—1·1	—0·01	—1·1	0·00	0·0

* A representative sample was obtained thus:—The available lint was divided into eight equal parts. Portions from every part were taken in turn and mixed with every other part; the process being repeated a dozen times so that lint from each of the original parts was well distributed in a random manner in every other part. From all these thoroughly mixed parts small tufts were taken from different sides of each part and mixed together to form this sample.

We find therefrom that between Surat and Broach the mean length varies from 1·1 to 4·3 per cent for four cottons and 8·1 per cent for one cotton. Between Surat and Viramgam the variation is *nil* in three cases and 1·1 and 6·9 in two cases. Between Broach and Viramgam the variation is only 1·1 to 2·3 per cent for four cases and 8 per cent in one case. Further the differences are sometimes positive and sometimes negative and are also within experimental errors in most of the cases. Three exceptions where the observed differences are outside the experimental errors may be noted, *viz.*, B. D.-8 at Surat has greater mean length than at Viramgam and even than at Broach, where it was selected; and W.-8 × 1027, 11-4, which has an average length of 0·87 in. both at Surat and Viramgam shows a length of 0·94 in. in Broach.

We therefore conclude that there is no definite trend of variation in mean length (either towards lengthening or shortening) from one tract to another in general, though some variation may be expected in particular varieties like B. D.-8 which becomes shorter as we proceed from Surat through Broach to Viramgam.

2. *Distribution of fibre length.*

In Table II the frequency distribution of fibres according to their length is given.

TABLE II

Showing frequency distribution of fibre-length

1/8-in.	1027			B. D.-8			11-4			11-2			12-1			Remarks
	S	B	V	S	B	V	S	B	V	S	B	V	S	B	V	
2	0·8	..	0·5	..	..	0·5	0·9	..	0·7	..	..	0·7	..	..	0·5	
3	2·0	1·3	1·3	1·2	1·0	1·6	2·0	1·2	2·1	1·9	1·0	1·5	2·7	1·2	1·4	
4	2·9	2·1	2·0	1·5	2·0	2·1	3·2	1·9	3·1	3·1	2·0	2·2	3·7	1·5	1·8	
5	4·5	4·2	4·4	3·4	4·8	9·3	6·0	4·4	8·2	6·9	5·0	5·2	6·5	5·5	6·4	
6	9·3	12·2	11·4	10·7	15·8	16·3	16·5	11·6	19·7	15·0	17·2	15·9	15·0	16·0	18·5	
7	20·4	27·7	23·4	32·9	38·9	35·3	34·7	26·3	33·4	27·6	38·5	30·4	29·4	34·8	31·0	
8	31·2	36·1	32·8	36·2	29·8	24·2	26·8	34·6	22·1	28·3	24·3	27·5	26·8	25·4	26·5	
9	20·0	11·2	17·7	9·4	6·0	9·2	7·6	13·9	7·5	12·8	9·0	12·0	12·0	10·6	9·5	
10	7·4	3·7	4·7	3·3	1·2	1·5	2·3	4·1	3·2	3·4	2·0	3·3	2·8	3·5	2·8	
11	1·5	1·5	1·8	1·4	0·5	..	..	2·0	..	1·0	1·0	1·3	1·1	1·5	1·6	

S=Surat, B=Broach, V=Viramgam.

We notice in Table II that if we take the modal group for any type in the three tracts, the Broach sample has the maximum percentage of fibres. For instance for 1027, 8 is the modal group for all the tracts ; and in this Broach has a maximum of 36·1 per cent, Surat 31·2 per cent and Viramgam 32·8 per cent of fibres. Except for 11-4, where there is practically no difference among the tracts, Surat and Viramgam are similar while Broach has a greater percentage. A maximum difference of 10·9 is found in the modal value between Broach and Surat for 11-2.

The frequency curves for these distributions are given in Fig. 1. All the curves are practically of the same type and there is considerable overlapping of the curves for any sample from the three tracts. This is particularly noticeable in the case of B. D.-8, 1027, 12-1 and 11-4. In 11-2 the Broach curve stands prominent with a sharp peak. Even though the variations in mean length are not significant these curves show that the percentage distribution of fibres of different lengths is slightly altered from tract to tract. This is well brought out when the figures are compared from group to group.

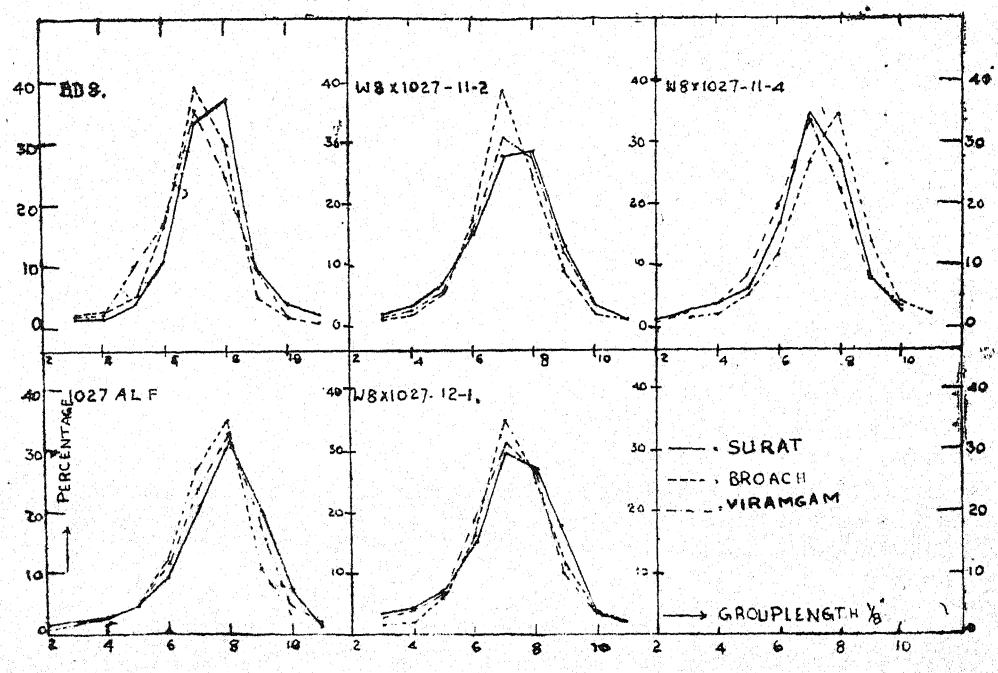

Fig. 1. Fibre-length tribution.

3. *Irregularity percentage*

From the frequency-distribution table we can calculate the irregularity percentage which, as is shown by Ahmad [1933, 2] is a statistical measure giving the percentage of fibres less than three-fourths of the modal length. Obviously the less the percentage the greater the spinning quality, all else remaining same. Table III below gives the percentages for all the samples.

TABLE III

Irregularity percentage and mean differences

No.	Sample	Surat S	Broach B	Viram-gam V	S—B	V—B	V—S
1	2	3	4	5	6	7	8
1	1027 A. L. F. . .	15·0	12·1	14·8	2·9	2·7	—0·2
2	B. D.-8 . . .	10·7	7·0	10·5	3·1	2·9	—0·2
3	W.-8 × 1027 11-4 .	13·3	12·0	13·8	1·3	1·8	0·5
4	W.-8 × 1027 11-2 .	15·1	7·5	12·0	7·6	4·5	—3·1
5	W.-8 × 1027 12-1 .	15·5	8·3	13·0	7·2	4·7	—2·5

Between Surat and Viramgam the percentage in any one type differs from —3·1 to plus 0·5, a negligible difference, between Surat and Broach it varies between 1·3 and 7·6 per cent and between Viramgam and Broach 1·8 to 4·7 per cent. In the latter two cases even though the differences are not very great for all types, it is uni-directional and in two cases more than 7 per cent. An error of two per cent may in general be assumed to occur in the determination of this property and thus the difference between Viramgam and Surat becomes insignificant except for 11-2 and 12-1. From columns 6 and 7 of the above table we also find that with the exception of 11-4, all the differences between Broach and either Surat or Viramgam are outside the experimental error of two per cent, the Broach samples always having a lower irregularity percentage. Besides, for 11-2 and 12-1 the differences between Surat and Broach are greater than thrice this error and are even statistically significant.

From these facts we may infer that the samples grown in Broach are more regular in staple than those grown either at Viramgam or Surat, except for 11-4 which shows a very small difference; while there is no difference in this respect between Surat and Viramgam except for 11-2 and 12-1.

4. Fibre-weight per inch

This in general is a quantitative measure of the fineness of a sample. Table IV below gives the results of fibre-weight per inch for all the samples in 10^{-7} ozs.

TABLE IV

Fibre-weight per inch and percentage mean differences

No.	Sample	Surat S	Broach B	Viram-gam V	S—B	$\frac{S-B}{S}\times\frac{100}{1}$	V—B	$\frac{V-B}{V}\times\frac{100}{1}$	V—S	$\frac{V-S}{V}\times\frac{100}{1}$
1	1027 A. L. F. .	1·58	1·57	2·07	0·01	0·6	0·50	24·2	0·49	23·7
2	B. D.-8 . . .	1·71	1·56	1·92	0·15	8·8	0·36	18·8	0·21	10·9
3	W.-8×1027 11-4 .	1·84	1·52	1·97	0·32	17·4	0·45	22·8	0·13	6·6
4	W.-8×1027 11-2 .	1·86	1·64	2·00	0·22	11·8	0·36	18·0	0·14	7·0
5	W.-8×1027 12-1 .	1·49	1·43	1·80	0·06	4·0	0·37	20·6	0·31	17·2

The fibre-weight for any one sample varies from 0·01 to 0·32 units between Surat and Broach, from 0·36 to 0·50 between Viramgam and Broach and from 0·13 to 0·49 between Surat and Viramgam. Expressed as percentages, Broach samples are finer than either Surat or Viramgam by 0·6 to 17·4 per cent and 18·0 to 24·2 per cent, respectively. The Surat samples are finer than Viramgam ones by 6·6 to 23·7 per cent. Since the maximum error in the fibre-weight determination would be about six per cent, most of these differences are statistically significant It therefore appears that in general these types have a graded variation from tract to tract ; though the amount of this variation is different for different types, each reacting differentially to the internal and external environmental factors of soil and atmosphere respectively, depending upon their inherent capacities. The effect of tract is most pronounced for coarseness in case of 1027 as between Viramgam, and Broach or Surat, while it is practically *nil* as between Surat and Broach. Between Broach and Viramgam the differences are particularly high and roughly uniform for all cottons, varying in percentage from 18·0 to 24·2. 11-2 and 11-4 have a markedly higher fibre weight in Surat than in Broach.

Judging the results as a whole we conclude that the types are coarsest in Viramgam and finest in Broach, Su at being assigned an intermediate position.

5. Fibre-maturity

As has been pointed out earlier the genesis of the present investigation lies in the observed differences in this property. Table V below gives the maturity results for all the samples. The first number gives the percentage of mature hairs and the second one percentage of dead hairs. The difference between the sum of these two and 100 giving the percentage of half-ripe fibres.

TABLE V

Fibre-maturity percentage and mean differences

No.	Sample	Surat S	Broach B	Viram-gam V	S—B	V—B	V—S
1	1027 A. L. F. . .	75—8	56—9	80—6	19—$\bar{1}$	24—$\bar{3}$	5—$\bar{2}$
2	B. D.-8 . . .	86—3	70—6	90—1	16—$\bar{3}$	20—$\bar{5}$	4—$\bar{2}$
3	W.-8×1027 11-4 .	58—7	57—11	76—4	1—$\bar{4}$	19—$\bar{7}$	18—$\bar{3}$
4	W.-8×1027 11-2 .	76—5	63—8	79—4	13—$\bar{3}$	16—$\bar{4}$	3—$\bar{1}$
5	W.-8×1027 12-1 .	65—9	61—6	73—5	4—3	12—$\bar{1}$	8—$\bar{4}$

$\bar{1}$ = minus 1.

Considering first the percentage of dead hairs we note that the differences between any two tracts or samples themselves are very small and we may hence conclude that there is no variation in this respect. Considering the percentage of mature hairs, we find that in every type there is a gradation from tract to tract. Compared with Surat, Broach has a lower percentage varying from one to nineteen and Viramgam a higher percentage ranging from three to eighteen; and Viramgam has twelve to twenty-four per cent more mature hairs than Broach. In this test a difference of eight per cent and more in any one class of hairs is considered significant. Applying this criterion we find that as between Viramgam and Surat the maturity difference is significant for only 11-4. It is significant for three samples 1027, B. D.-8 and 11-2 as between Broach and Surat, while it is significant for all cottons as between Broach and Viramgam. Thus the difference in the degree of hair-maturity varies from tract to tract as between cotton and cotton.

These differences, which are significant, lead us to conclude that the Broach samples are the least mature, Viramgam samples containing the highest percentage of mature hairs, while the Surat samples occupy intermediate positions. The differences which are not significant are however also in the same direction as the significant ones. This variation which is uni-directional goes hand in hand with the fibre-weight results noted earlier. The tract with a maximum fibre-weight has a maximum maturity and the finest variety tract the lowest maturity, and the third one intermediate in both cases. It must be noted however that these are relative differences among these three tracts only.

One more point emerges from the correlation of these two results. The facts that the finest (Broach) is least mature and, conversely, the coarsest (Viramgam) most mature taken in conjunction with each other, indicate that **relatively** the fibres will be found weakest in Broach and strongest in Viramgam.

These results obtained for three different types indicate that we may possibly expect similar results to hold good for another type when transferred from one tract to another provided the season is a normal one, because tract includes (*i*) soil, and (*ii*) external environment. Soil is generally same in a tract and thus the changes are truly reflected provided there is no abnormality in the external conditions confined to only one tract. Given a normal season, when no particular tract was singled out for abnormal atmospheric conditions, the five samples tested gave results leading to the following conclusions.

V. Conclusions

There is no significant variation in mean fibre-length of most of the types discussed above, when grown on the different farms of Broach, Surat and Viramgam. However, B. D.-8 tends towards decreasing mean length as we proceed from Surat through Broach to Viramgam, and strain 11-4 tends to be longer in Broach than either at Surat or Viramgam.

The irregularity percentage is least for Broach and similar as between Surat and Viramgam except for 11-2 and 12-1.

The fibre-weight per inch and hence the coarseness of samples is least in Broach, highest in Viramgam and intermediate in Surat, all the observed differences being beyond experimental errors except for 1027 and strain 12–1 as between Surat and Broach which however are in the same direction as the other differences.

Similarly the maturity percentage is maximum in Viramgam and minimum in Broach, Surat being intermediate. The differences are significant for all cottons as between Viramgam and Broach, for three as between Broach and Surat and for one as between Viramgam and Surat. The other differences are in the same direction as the significant ones.

The high maturity and high fibre-weight per inch in Viramgam and the low maturity and low fibre-weight per inch in Broach taken in conjunction with each other respectively indicate a probable **relative weakness** in Broach samples as compared with similar ones of Viramgam.

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DISEASES OF *CITRUS* IN THE PUNJAB

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(With Plates XII-XIX and one text-figure)

CONTENTS

	Page
I. Introduction	73
II. Wither-tip disease	
(A) *Anthracnose caused by Colletotrichum gloeosporioides Penz.*	75
(*a*) Review	75
(*b*) Symptoms	77
(*c*) Morphology	78
(*d*) Growth in culture	78
(*e*) Strains of *C. gloeosporioides*	81
(*f*) Effect of low temperature	83
(*g*) Heterothallism in *C. gloeosporioides*	84
(*h*) Method of penetration	84
(*i*) Pathogenicity	85
(*j*) Effect of stocks on scions regarding disease-resistance	89
Conclusion and summary	91
III. Other diseases of citrus	92
(B) *Chlorosis*—	92
(*a*) External symptoms	92
(*b*) Internal structure and chemical nature	93
(*c*) Control of chlorosis	94
Summary	95
(C) *Saprophytic moulds*—	95
(*a*) Symptoms	95
(*b*) *Acrothecium lunatum* Wak.	96
(*c*) *Capnodium citri* Berk. et Desm.	97
(*d*) *Alternaria citri* Pierce	97
(*e*) *Cladosporium herbarum*, var. *citricola* F. & B.	98
(*f*) *Chaetomium* sp.	98
(*g*) *Pleospora herbarum* Rab.	98
Conclusion and summary	98

IV. CONTROL MEASURES
(*a*) Control experiments at Lahore 99
(*b*) Control experiments at Nurpur 104
(*c*) Recommendation regarding spraying of fungicides 104
(*d*) Control of diseases by manuring and aerating 105
(*e*) General observations 106
Summary 107
Acknowledgments 107
REFERENCES 108

I. Introduction

Citrus plants in India suffer from a number of serious diseases, causing considerable losses, and very little has so far been done to study them with a view to controlling them. Lately, however, in several parts of India, the study of *Citrus* diseases has been undertaken. The important *Citrus*-growing provinces in India are Central Provinces, Assam, Punjab and Bombay. In Central Provinces, Bombay Presidency and in Assam mostly the loose-skinned mandarin group of oranges are grown, whereas in the Punjab, the close-skinned *malta* oranges form the most important *Citrus* fruits. Recently great strides have been made in *Citrus* growing in India. This has been mainly due, thanks to the Imperial Council of Agricultural Research, India, to better marketing facilities and horticultural experiments for introducing better varieties. The climatic conditions in all these provinces differ very much and so do the fruits. Lime (*Citrus aurantifolia*) is grown practically all over India and canker is almost universally associated with it, and is one of the most prevalent *Citrus* diseases. True scab, however, is very rare, but it has been definitely noticed in the Punjab and in Saharanpur Gardens and also in the South in the Madras Presidency. Anthracnose (wither-tip) due to *C. gloeosporioides* is found practically all over India but only in the Punjab it has become one of the most serious diseases of the *Citrus* plants. Die-back of orange plants in the Bombay Presidency associated with *Diplodia* sp. has been more often referred to as due to bad aeration, and is claimed to have been controlled [Sahasrabuddhe, 1927 ; Cheema and Bhatt, 1929] by proper aeration of the soil. A gummosis due to *Phytophthora palmivora* Butl. and a root-rot due to *Rosellinia* sp. have also been reported from Bombay Presidency besides anthracnose (*C. gloeosporioides*). Similarly from South India anthracnose and pink disease (*Corticium salmonicolor* B. et Br.) have been reported. The sooty mould is very common in many parts and generally follows in the wake of scale-insects. It may lower the vigour of the tree by completely and heavily encrusting all the leaves of the trees. In very bad cases, fruit-fall may be caused.

In the Punjab, almost all the varieties of *Citrus* fruits are to be found, but it is only the loose-skinned *santara* and the close-skinned *malta* oranges that are cultivated on a commercial scale. The lime (*nimbu*), citron (*galgal*), pomelo (*chakotra*), etc., are also being cultivated more and more. The sour orange (*khatta*), and the rough lemon (*khatti*) are extensively grown as hedge plants

throughout the Punjab. The rest are not grown on a commercial scale but are often found scattered individually in private gardens.

Throughout this country there is great confusion about the identity and classification of the *Citrus* varieties. Different botanists have formulated different classifications, establishing the species and varieties on not very reliable and stable characters with the result that the calssification of *Citrus* plants is in a state of hopeless confusion. This confusion has become worse owing to the readiness with which several species hybridise and also perhaps, as Bonavia [1888] says, owing to changes brought about in a new environment when plants are first introduced. Brandis [1874], Bonavia [1888], Lushington [1910], Swingle [1914], Coit [1915], Fawcett and Rendle [1920], Davis [1924], Parker [1924], Hume [1926], Tanaka [1927, 1930] and others have given different classifications and descriptions with the result that the selection of the correct botanical name is extremely difficult. Recently Cheema and Bhatt [1934] have studied the *Citrus* varieties of the Bombay Presidency and have recommended Swingle's classification [1914] for adoption. The confusion that at present prevails may be seen from the following different botanical names that have been applied by different botanists to the loose-skinned *santara* orange, *viz.*, *Citrus nobilis* Lour ; *C. nobilis* (Lour.) var. *deliciosa* Swing. ; *C. deliciosa* Tenore ; *C. poonensis* Tanaka ; and *C. aurantium sinense* Rumph.

In this paper the vernacular names as used in the Punjab, have been used throughout, for the work was undertaken with a view to help the *Citrus* growers of the Punjab and the references to the plants must be easily understood by them.

Advantage was taken of Professor Tanaka's recent visit to Lahore. The author had all the local varieties examined and named by him and the following list shows the vernacular names, with their botanical and nearest English equivalents, referred to in this paper. He is greatly indebted to Prof. Tanaka for this.

Vernacular name	Botanical name	Nearest English equivalent
Malta . . .	*Citrus sinensis*	Sweet orange
	NOTE.—Introduced from Europe into Gujranwala by Col. Clark, 1852-56. The Gujranwala blood-red oranges are still famous throughout the province.	
Santara . .	*Citrus poonensis*	Mandarin type
Mitha . . .	*Citrus limetta*	Sweet lime
Khatti (Gambhiri) .	*Citrus jambhiri*	Rough lemon
Khatta . . .	*Citrus aurantium*	Sour orange
Chakotra . .	*Citrus grandis*	Shaddock or Pomelo
Grape fruit . .	*Citrus paradisi*	Grape fruit
Galgal (big) . .	*Citrus medica*	Coarse citron
Galgal (small) .	*Citrus limon* var.	Lemon type
Nimbu (lemon) .	*Citrus limon*	Lemon
Nimbu (Kagzi) .	*Citrus aurantifolia*	Lime

The following diseases of the *Citrus* plants occurring in the Punjab, *viz.*, the 'wither-tip,' (*Colletotrichum gloeosporioides* Penz.), sooty mould and chlorosis are dealt with in this paper. The anthracnose (wither-tip) causes the greatest loss to the *Citrus* in the Punjab, and this disease has now been studied in detail and remedial measures have been suggested.

II. Wither-tip disease

(A) Anthracnose (wither-tip) caused by *Colletotrichum gloeosporioides* Penz.

(*a*) *Review*

This disease is found throughout the province and can be seen on most of the *Citrus* species, though it is more common on *santara* and *malta* (Plate XVI, figs. 1, 2). The diseased tips become dead and assume a silvery grey appearance on which minute dark acervuli of *Colletotrichum* soon appear. The healthy portion becomes sharply demarcated from the diseased portion (Plates XV). The disease, however, is not equally severe in all parts of the province and has been noticed in the following places, *viz.*, Peshawar, Wah, Rawalpindi, Jammu, Udhampur, Pathankot, Nurpur, Kangra, Baijnath, Jullundur, Hoshiarpur, Saharanpur, Delhi, Patiala, Lahore, Amritsar, Gujranwala, Daska, Ranbirsinghpura, Udera, Mangtanwala, Sheikhupura, Sarakpura, Bahawalpur, Renala, Multan and Dehra Gazi Khan (Fig. 1.) It has been noticed that generally along the low hills and places within a hundred miles from the foot of the hills the most badly affected orchards are found. But it has been felt all the same that the prevalence of the disease in an epidemic form is due more to ignorance and neglect than to anything else. For instance, in the Gujranwala-Daska area which is a big *Citrus*-growing centre with a large number of old and new plantations, the wither-tip disease prevails in a rather severe form throughout. In many of the old gardens dying or dead plants are left in the field which act as a perpetual source of infection to the other healthy plants. In the newer orchards, two or three years old, most of the plants were found to be already affected. This was primarily due to the fact that the growers not having any idea about the disease started with diseased stocks, and, in two or three years' time when they would expect the yield, perhaps many of the orchards would have to be cut down altogether after about five years of ignorant care and attention and a loss of good deal of money and energy. This will no doubt be a great set-back to the growing industry. It is difficult to find nurseries in the Punjab free from wither-tip and canker diseases. Chaudhuri and Singh [1930] found the anthracnose organism (*C. gloeosporioides* Penz.) in many parts of India, but nowhere it occurred in such epidemic form as in the Punjab. Though it has often been looked upon as causing a secondary infection, there is no doubt that in many parts of the

world it occurs as a serious parasite of *Citrus* plants and causes great loss. Rolfs [1904], Essig [1911], Barrett [1915] and others, and in recent years Burger [1923], Peyronel [1926], Reichert [1928], Muller [1933] and others have found it parasitic on different *Citrus* species. Petri [1926] proved its parasitic nature on lemon by means of inoculation experiments. He also [1927] considered it to be the cause of *mal secco*—a very serious disease of *Citrus* in Italy. Later however [1928, 1929], he doubted its being the cuase and found [1926; 1930, 1; 1930, 2; 1931] *mal secco* to be due to a new fungus which he named *Deuterophoma tracheiphila*. The two fungi are very often associated, and Petri [1930, 2] has compared the characteristics of the two. Savastano and Fawcett [1930] and others have recently confirmed Petri's diagnosis regarding *mal secco*.

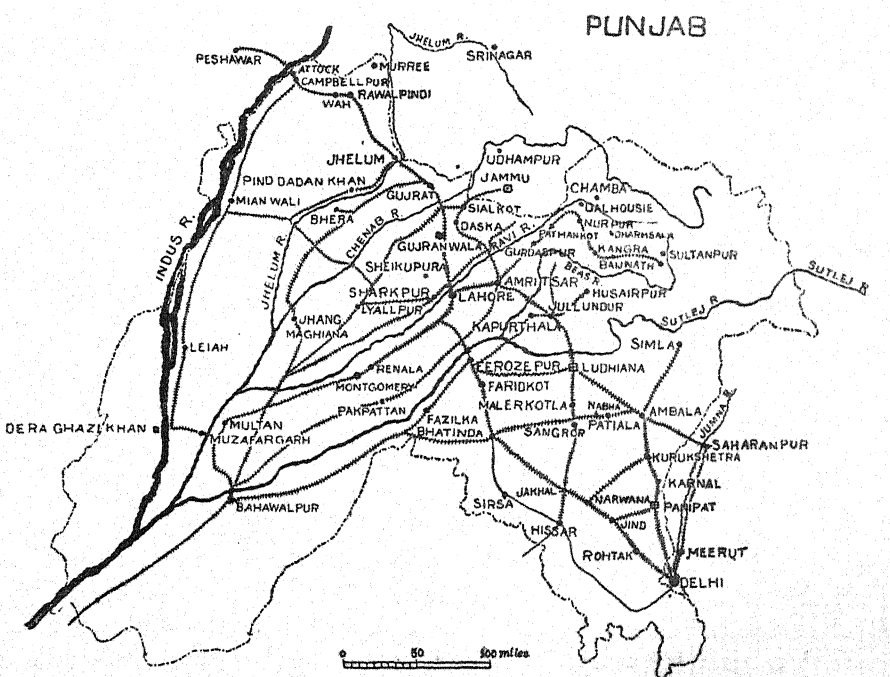

Fig. 1. Map of the Punjab showing places where wither-tip of the *Citrus* has been noticed.

Another similar anthracnose-producing organism, *Gloeosporium limetticolum* Claus. frequently causes serious damage to limes in Florida, Cuba and West Indian Islands, and it has been found by Fulton [1925] and others that in those countries *C. gloeosporioides* Penz. only attacks later on when the plants have been weakened by the attack of *Gloeosporium*. A serious wither-tip disease of limes from Dominica, due to *G. limetticolum* and causing very similar symptoms to *C. gloeosporioides* wither-tip, has been described by Stell [1924], Wiltshire [1925] and Ashby [1925]. It threatens to destroy the crop in some parts of the

island. The lime wither-tip was first described by Watts [1923] as being due to *C. gloeosporioides*. In fact the two organisms are very much alike. In *C. gloeosporioides* Penz. the pustules are frequently devoid of setae and in that condition it is difficult to distinguish it from *G. limetticolum* Claus. Petri [1927] also found that the setae are not regularly produced in the acervuli and often are completely absent. *C. gloeosporioides* is very variable [Burger, 1921] and several strains which differ in their virulence have been found. Chaudhuri and Singh [1930] and Muller [1933] isolated distinct forms from different *Citrus* species.

Though the fungus is quite commonly found on dead twigs of *Citrus*, which may have been killed by other organisms or by any other cause, there is no doubt, however, about its power of causing disease unaided, as has been proved by a large number of inoculation experiments. It has been very frequently observed, as mentioned by Fawcett [1915], that some of the severest injuries in connection with this disease may be due to the combined effect of anthracnose fungus and other fungi, such as *Diplodia*, *Phomopsis*, etc. Also trees previously weakened by unfavourable soil conditions, such as continued low temperature, excessive frosts, etc., become more susceptible to this disease. In 1929 great loss occurred when, for three successive days during January, Lahore experienced a cold wave and had 15 to 20° of frosts everyday. The *Citrus* plants were chilled and weakened and shortly afterwards most of the plants in the nurseries near-about were attacked and killed by the wither-tip organisms. It has also been noticed that injuries produced by a severe hail-storm made the entry of the wither-tip organisms easy. In Plate XIX, fig. 1 a twig which had been injured by hail-storm and later became infected with *C. gloeosporioides* Penz. is depicted. Barrett [1922] reported anthracnose on fruits when injured by hail-stones.

The wither-tip of *Citrus*, though a minor disease in other parts of India, has become a major one in the Punjab. There is nothing to be wondered at in this. For it is a well-known fact that " the differences in climate and other conditions between two countries or even two localities in the same country, may be so great as to make diseases that are major in one place, minor or of no economic importance in the other. Major diseases of one variety may be minor in another " [Fawcett and Lee, 1926]. Even in the Punjab it has been noticed that the virulence of the disease differs in different parts of the province, which however may be due to having several strains of *C. gloeosporioides*, some being more vigorous than the others.

(*b*) *Symptoms*

In Plate XV twigs of *Citrus* affected by wither-tip due to *Colletotrichum gloeosporioides* are shown. The sharp demarcation of the diseased portion from the healthy part and their silvery-grey appearance are very characteristic. The acervuli of the fungus may be seen as black dots scattered throughout the dead part of the twig (Plate XV). In moist weather these may be seen bursting when

pinkish masses of spores are seen oozing out. The setae may be seen frequently around these bodies. These may be either continuous or septate. In the anthracnose spots of the leaves, the acervuli are found on the upper surface and are half submerged in the tissue of the leaf (Plate XIX, fig. 2).

(c) *Morphology*

Acervuli and spores.—The diameter of the acervuli ranges from 60 to 270μ. The size of the spores varies a good deal and even in the same acervulus a lot of variation in spore sizes is seen. On measuring one hundred spores at random the length of the spores has been found to vary between 11·2 and 21·0μ and the width between 2·4 and 7·0μ. The mean values of length and breadth of spores from a malta twig were found to be 13·2 and 5·5 μ, respectively.

Spores are somewhat greenish when young and are full of protoplasmic granules and oil globules. Later they become somewhat hyaline. When mature, spores ooze out in mass from the acervuli; they appear pinkish in colour. They have usually one nucleus but frequently two nuclei have also been noted. Spores germinate readily. In hanging drops germination was observed in four hours. Germ-tubes are usually formed from one end though sometimes they may be formed from both the ends and the hyphae produced may be seen forming conidia in four to five days' time (Plate XII, *c*, *d*, *e*). The apex of the conidiophore becomes swollen, into which protoplasmic granules, etc., pass and become cut off from the rest of the conidiophore, thus forming a conidium. When it is abstricted, another conidium will be formed in the same way. Sometimes when a number of spores are put in a hanging drop, it is common to find the spores become septate at the time of germination by the formation of a partition wall.

(d) *Growth in culture*

Single spore cultures were taken in this study. It grows well on several media such as potato glucose and potato extract media, pea, bean, prune, gram, wheat and barley agar and also on Coons', Claussen's, Barnes', Raulin's, Czapek's and Richards' media. In the preparation of the first series of media, excepting in potato glucose, five grms. of materials were taken for every 100 c.c. of water and two per cent of agar was added. For prune agar, more agar was added if necessary. Potato glucose agar and the different synthetic media were prepared in the usual way. Petri dishes of uniform size (90 mm.) were used and 20 c.c. of the media poured to have a uniform thickness. In Table I, the growth characters and the total spread of the fungus isolated from a diseased *malta* twig have been shown in some of the above media.

TABLE I

Growth character and spread of C. gloeosporioides *in different media at room temperature*

Media	Growth character at room temperature (about 26°)	Spread in six days (diameter in mm.)
Pea agar . . .	Cottony hyphae, not very luxuriant, acervuli produced in ring which soon burst oozing out pink mass of spores. No blackening of reverse surface.	54
Bean agar . .	Woolly hyphae, aerial hyphae erect. Pinkish spore masses appear early only near the centre, reverse side not blackened.	45
Potato glucose agar .	White fluffy growth, reverse darkened, acervuli appear late, formed near centre.	45
Prune agar . .	Profuse aerial growth, white at first, later somewhat greyish and finally almost black. Many big-sized acervuli. Reverse blackened.	60
Barley agar . .	Hyphae poorly developed, moderate, acervuli with setae, usually in rings. Reverse side becomes blackned later.	35
Maize agar . .	Aerial growth poor, acervuli few . . .	47
Czapek's agar . .	Dense woolly growth, limited number of acervuli, reverse side turns black.	45
Coons' agar . .	Scanty mycelial growth, reverse almost transparent, limited minute acervuli in a ring.	45
Richards' agar . .	Aerial hyphae almost absent, many acervuli .	62
Dox' agar . .	Dark radiating hyphae, reverse almost black, a few acervuli in centre.	55

In Plate XVII, figs. 1-4, the growth characters in potato glucose, prune, Coons' and Dox' media are shown.

The length and breadth of the spores on different media varied a good deal and the smallest sized spores were found when cultures were grown in plain agar. The mean values for length and breadth, using 100 spores, are given in Table II.

TABLE II.

Mean values of length and breadth in μ of C. gloeosporioides *spores in certain media*

Media	Length	Breadth
Plain agar	9·6	3·8
Barley agar	12·3	4·2
Pea agar	13·5	4·5
Potato agar	15·2	5·0
Raulin's agar	15·1	5·0
Richards' agar	16·0	4·0
Barnes' agar	18·8	4·9

Since the growth character and the size of the spores were found to depend on the media employed, it was decided to grow the fungus on different concentrations of media and to study the effect of concentration on growth. For this two synthetic media, Richards' and Czapek's were selected. Petri dishes were inoculated with a spore suspension placed in the centre and these were incubated at 25°C.

It has been found that the greatest spread of the mycelium takes place at normal concentrations of the two media and that it decreases regularly either way when the concentrations are increased or decreased. Regarding the length

of the spores, it has been found that in both the media it increases from the lower to the higher concentrations. No such regularity was observed regarding width. Though the length of the spores increased in higher concentrations, the number of spores found in very high concentrations was fewer and at 4*N* in Czapek's no spores were found in nine days' time.

(*e*) *Strains of* C. gloeosporioides

Chaudhuri and Singh [1930] mentioned the possible existence of strains, and referred to three probable strains isolated from different *Citrus* species which showed difference in spore measurements. Though a large number of cross-inoculations were done, each strain succeeded in infecting all the other hosts. Beside slight morphological differences, no physiological differentiation was found. This work has now further been extended and four different strains from different hosts have been isolated which show decided difference in their virulence and growth characters. There are possibly many other strains. The detailed descriptions of the four strains isolated have been given later. The difference in the size of the spores is not a fundamental criterion in determining a strain without considering various other characters. As has already been shown the spore-size not only varies with difference in the kind of nutrient media on which it is grown, but also depends on temperature, and it has been found in nature that even on the same host, the size of the spore varies in different seasons. The average spore sizes (counts of fifty) in microns from different varieties in two seasons are given below :—

	September.	May.
Musumbi	13·7 × 4·1	14·4 × 3·9
Excellence	14·5 × 4·1	14·9 × 3·7
Italian	14·0 × 4·2	14·0 × 4·5
Blood Red	13·6 × 4·0	14·6 × 3·9
Jaffa	14·5 × 4·2	12·9 × 3·8

The average measurements of spores (counts of fifty) on potato glucose agar at room temperature from different plants in the first generation of culture were taken and those forms showing difference in spore-measurements were first grown under different physiological conditions for selecting the strains. From these only those showing permanent differences were chosen.

In Table III, the total amount of spread of hyphae in three different media and variation in spore measurements of certain of the above forms isolated from *malta*, *santara*, *khatta*, lemon, *galgal* and *mitha* have been shown. Culture from each has been grown at two different temperatures, *viz.*, 25°C. and 30°C. and the difference in the total spread and spore size at these two temperatures will be seen.

TABLE III

Effect of different media and temperatures on the spread and spore size of C. gloeosporioides *isolated from different host plants*

Host	Temperature (° C.)	Maize extract agar		Potato glucose agar		Czapek's agar	
		Spread in mm.	Spore size (average o 50) in μ	Spread in mm.	Spore size (average of 50) in μ	Spread in mm.	Spore size (average of 50) in μ
Malta	25°	60	14·3 × 5·2	55	12·7 × 4·1	70	13·2 × 4·2
	30°	70	13·5 × 5·3	70	12·1 × 4·1	70	14·2 × 4·2
Santara	25°	60	11·6 × 4·0	67	11·8 × 4·0	70	15·5 × 3·9
	30°	72	12·5 × 4·6	80	14·6 × 3·7	72	14·2 × 4·7
Khatta	25°	63	14·8 × 5·0	66	13·4 × 4·8	73	12·3 × 3·8
	30°	80	13·2 × 4·2	80	14·5 × 4·4	74	11·7 × 3·9
Lemon	25°	60	13·3 × 4·6	64	...	70	12·5 × 3·2
	30°	70	11·6 × 3·8	70	...	70	12·2 × 3·7
Galgal	25°	59	12·5 × 4·6	64	11·2 × 4·1	70	12·3 × 3·4
	30°	75	12·3 × 5·2	80	...	76	13·8 × 4·2
Mitha	25°	62	11·2 × 3·8	70	11·4 × 3·9	70	...
	30°	70	10·2 × 4·7	77	14·0 × 4·7	77	

From the large number of parallel cutures grown and from the study of their behaviour under different physiological conditions strains A to L were isolated, and of these the following four were found to have distinctive characters, *viz.*—

Strain A . . . On *santara* from Renala Khurd (culture No. 8·9/7)
Strain B . . . On *malta* from Lahore gardens (culture No. 1·9/24)
Strain D . . . On *malta* from Lahore gardens (culture No. 1·9/15)
Strain F . . . On *santara* from Lahore gardens (culture No. 15·8/19)

The above strains have been grown on oatmeal, rice agar and Czapek's agar, and their growth characters at 26°C. are given in Table IV.

TABLE IV

Growth character of four strains of C. gloeosporioides *on different media*

Strains	Oatmeal agar	Ricemeal agar	Czapeks' agar
Strain A. No. 316.	Mycelial growth poor, many acervuli, almost colourless, appear a little late. Spread 70 mm. in 7 days.	Very scanty colourless hyphæ at first—later blackish—central portion fluffy; black radiating hyphæ when viewed from below. Big sized hyphal knots seen. Spread 70 mm. in 7 days.	White fluffy growth; bottom—pink to dark gradually; central dark portion with plenty of acervuli. Spread 70 mm. in 7 days.
Strain B. No. 345.	Growth poor but largest number of minute acervuli formed. They appear early and develop in concentric rings. Clear from below. Spread 71 mm.	Growth whitish with dark hyphæ radiating in between (saltating ?). No acervuli in 7 days. Spread 66 mm.	Very white fluffy growth; Viewed from below appears blackish. Acervuli formed late, spread 70 mm.
Strain D. No. 347.	Growth poor but large number of acervuli formed later. Spread 71 mm.	Growth scanty—blackish radiating hyphæ seen when viewed from below; small hyphal knots. No acervuli in 7 days, but formed very late. Spread 65 mm.	White fluffy growth. Acervuli appear very late. Spread 72 mm.
Strain F. No. 233.	Cottony hyphæ; only a few acervuli developed. Whitish from below. Spread 72 mm.	White cottony growth; no radiating blackish hyphæ seen from below; only a few scattered acervuli formed very late. Spread 63 mm.	Fluffy white radiating; bottom pinkish to dark No acervuli in a week. Spread 73 mm.

From the above it will be seen that though the media determine the character of the growth to a great extent, the above four strains retain their distinctness in all these media, and may be taken as different strains.

(*f*) *Effect of exposure to low temperature*

Effect of very low temperature on the four different strains was observed. Five-day old cultures in oatmeal, rice and Czapek's agar in duplicate of each strain were placed in a refrigerator at 0°C. for a period of one month and twenty

days. Then these were transferred to an incubator at 25°C. All these revived. The causal organisms are not killed by prolonged exposure to very low temperature.

(*g*) *Heterothallism in* C. gloeosporioides

All the cultures A to L have been grown in groups in different Petri dishes to see how the two cultures behave when the hyphæ of one come in contact with the hyphæ of another, *viz.*, whether they mix together or form a space of aversion or a white line of aversion.

In all these cases monospore cultures have been used as stated before, and it has been noticed that certain cultures mix freely when put together, in others a space of aversion is formed between the two, while in some other cases a white line of aversion is formed. These results show definitely heterothallism in this fungus. Though all combinations have been tried, no fruit-bodies have been made to form yet. Complete fusions were seen between E-C, F-D, B-D, B-H, J-K, F-C, B-H, E-G.

Space of aversion between B-E, A-G, G-B, E-A, A-D, K-L, L-I, L-J, C-D, A-C, C-D, H-C, C-G, C-H, B-F, D-F, A-G.

White line of aversion between B-F, A-B, C-E, A-B, A-F, G-F, B-D, C-E.

In Plate XVII, figs. 5, 6 complete fusions, spaces of aversion and white lines of aversion between certain strains are shown.

(*h*) *Method of penetration*

The method of infection by *C. gloeospo rioides* hasbeen studied on *malta* leaves. Spore suspensions were placed on the upper surface of the leaves. The spores on germination produce appressoria-like structures which stick fast to the surface of the leaves. These appressoria are very regular in shape and size and are able to withstand desiccation and should be looked upon as chlamydospores. The appressorium in *Gloeosporium* has also been described as a chlamydospore by Biraghi [1934]. When the spores germinate, they give out certain products which react on the cuticle and the plasma membrane and the cells below get injected before actual penetration takes place. The stimulus to germination of the appressoria might be supplied by nutrient substances diffusing out when cells get injected. Injection has been produced by placing filtered drops in which spores were germinating for thirty to forty hours and when later the leaf was flooded with a dilute solution of erythrosine, the dye was absorbed by the injected spots only. No fine peg-like infection hyphæ as noticed by Dey [1933] have been noticed but instead ordinary germ-tube like structures have been seen to penetrate.

The details regarding the method of penetration have been published by the author in a separate paper [Chaudhuri, 1935].

(i) *Pathogenicity*

Cultures of wither-tip organisms from *khatta* (*C. aurantium*), *malta* (*C. sinensis*) and *santara* (*C. poonensis*), as well as spores from nature were used for inoculation experiments. In the first series of experiments only cut leaves were used. These were washed in 0·01 per cent mercuric chloride solution, and after carefully washing in several changes of distilled water, were placed in sterilised Petri dishes containing moist cotton wool. These were now sprayed with spore suspension and incubated at 27°C. Table V shows the result of experiment after a week's observation.

TABLE V

Result of inoculation of malta, santara *and* khatta *leaves with* C. gloeosporioides *spores found on those host-plants as well as with spores formed in culture from them*

Fungus strain	Host	No. of leaves sprayed	No. taking infection	No. of control	No. of control infected	Remarks
Malta wither-tip spores from culture	*Malta* leaves	6	6	2	*nil*	Many acervuli on upper surface of inoculated leaves.
Do. spores naturally formed	Do.	6	6	2	*nil*	Several spots with acervuli on upper surface of inoculated leaves.
Santara wither-tip spores from culture	*Santara* leaves	6	6	2	*nil*	Brownish spots, white mycelial growth, acervuli on both sides of inoculated leaves.
Do. spores naturally formed	Do.	6	6	2	*nil*	As above, acervuli bursting.
Khatta wither-tip spores from culture	*Khatta* leaves	6	6	2	*nil*	Brownish spots on inoculated leaves but no acervuli formed yet.
Do. spores naturally formed	Do.	6	6	2	*nil*	As above

Plate XVI, fig. 4 shows innumerable acervuli formed on a *malta* leaf when sprayed with *malta* wither-tip spore suspension in distilled water.

When the above experiment was performed at 15°C. instead of at 27°C., no change was visible for a week but when the Petri dishes were removed after that to a higher temperature (27°C.) quick changes were observed. Three days after removal to a higher temperature, positive infection was found on *malta* and *santara* leaves (whether sprayed with naturally formed spores or with spores formed in culture) but no infection was found on *khatta* leaves. Control leaves were all healthy. Table VI shows the results of cross-inoculation experiments with cut leaves in a tabulated form. Certain marked spots on the leaves were pricked with a sterilised lancet needle after the leaves had been washed in 0·01 per cent mercuric chloride solution and distilled water. Cultures of *malta* (N. S. 235), *pomelo* (N. S. 237), *santara* (N. S. 233) and orange (*Citrus aurantium* C. S. 233) wither-tip have been used. The experiments were all done at room temperature (25°—30°C.).

TABLE VI

Result of experiment using cut leaves

Host	Observation after		Remarks
	8 days	12 days	
(*a*) *Inoculated with spores from* malta *wither-tip* (*N. S. 235*)			
Orange (Assam)	Spots pale green .	Spots pale brown	No acervuli formed. No infection.
Khatta . .	No change . .	Spots pale yellow	Acervuli formed. Positive infection.
Pomelo . .	Spots pale brown	Spots pale brown	No acervuli. No infection.
Malta . .	No change . .	Spots yellowish brown.	Acervuli formed, some bursting, positive infection.
Lemon . .	Spots pale brown	Spots pale brown	Many acervuli, some bursting, positive infection.
Mitha . .	Spots pale brown	Spots pale brown.	A few acervuli formed, positive infection.
Galgal . .	Spots pale yellow.	Spots brownish .	No acervuli, no infection.

Host	Observation after		Remarks
	8 days	12 days	
(*b*) *Inoculated with spores from* pomelo (*N. S. 237*)			
Mitha . .	Spots brownish .	Spots dark brown	No acervuli formed, no infection.
Malta . .	Spots pale brown	Spots deep brown	Pinkish spore masses coming out of acervuli. Positive infection.
Santara . .	Spots pale green .	Spots yellowish .	Acervuli formed, positive infection.
Galgal . .	As above . .	As above . .	As above
Khatta . .	As above . .	As above . .	As above
Lemon . .	As above . .	As above . .	As above
Pomelo . .	As above . .	As above . .	Innumerable acervuli formed, positive infection.
(*c*) *Inoculated with spores from* santara (*N. S. 233*)			
Santara . .	Spots pale green .	Spots pale brown	Innumerable acervuli from which pinkish spore masses are oozing out, positive infection.
Orange (Assam)	Spots pale green .	Spots pale green .	No acervuli, no infection.
Pomelo . .	Spots pale green .	Spots dark brown	Acervuli formed.
Mitha	Spots pale green .	Spots pale brown	No acervuli formed, no infection.
Galgal . .	Spots pale brown	Spots dark brown	Acervuli oozing out, pink spore masses early. Positive infection.
Malta . .	Spots pale green .	Spots brownish .	Many acervuli, pinkish spore masses oozing out, positive infection.
Khatta . .	Spots pale brown	Spots dark brown	Acervuli formed, spore masses oozing out, positive infection.

Host	Observation		Remarks
	8 days	12 days	
(d) Inoculated with spores from orange (C. S. 233)			
C. trifoliata .	Spots pale brown	Spots dark brown	No acervuli, no infection.
Orange (Assam)	Spots pale yellow.	Spots brownish .	As above
Galgal . .	Spots pale brown	Spots dark brown	A few acervuli, positive infection.
Pomelo . .	Spots pale brown.	Spots dark brown	Acervuli formed, positive infection.
Mitha . .	Spots pale brown.	Spots brownish .	No acervuli, no infection.
Khatta . .	Spots pale brown.	Spots dark brown	A few acervuli, positive infection.

Table VII shows the result of inoculation with cultures isolated from wither-tip of orange (233 C. S. and 216 C. S.), *malta* (235 C. S.), *pomelo* (238 C. S.) and from a local *malta* leaf (C. S. 230) on different varieties of *malta*. The twigs were obtained from the Indian Mildura Fruit Farm, Renala Khurd.

TABLE VII

Result of inoculation of different varieties of malta *with spores from cultures isolated from different host-plants*

Varieties of *malta*	*Orange* 233 C. S.	*Malta* 235 C. S.	*Orange* leaf 216 C. S.	*Malta* leaf 230 C. S.	*Pomelo* 238 C. S.
	Acervuli				
Blood Red . . .	—	+	—	—	+
Wilkie	—	+	—	—	+
Excellence . . .	—	+	+	—	+
Italian	+	+	—	—	++
Jaffa	+	+	—	—	+
Musumbi	+	+	Few +	Few +	—
Ruby	—	+	Few +	Few +	++
Washington . . .	—	++	—	+	—

In Table VIII, the result of inoculation of potted *malta* and *s ant* plants with two different cultures of *malta* anthracnose and one of *santara* has been shown. Four plants were inoculated with each of these strains and two plants of each were kept as controls. The plants to be inoculated were pricked at several marked places (not on the leaves) near the tip and then sprayed with a thick suspension of spores. Shedding of leaves began after ten days and in a fortnight twigs showed signs of wither-tip. Control plants were all healthy.

TABLE VIII

Result of inoculation of potted plants with three different strains of C. gloeosporioides

Fungus strain	Host	No. of plants inoculated	Observation after two months
Malta wither-tip culture 22·7/7 of 347	*Malta* .	4	Three altogether dead, one shows disease.
Do.	*Santara* .	4	All leaves shed, tips show disease.
Malta wither-tip culture 19·7/1 of 345	*Malta* .	4	One dead, two twigs dying back showing wither-tip, one healthy.
Do. .	*Santara* .	4	Every twig shows wither-tip.
Santara wither-tip culture 22·7/6 of 346	*Malta* .	4	One dead, two diseased, one healthy.
Do. .	*Santara* .	4	Three very badly diseased, almost dead, one slightly affect.

From the above experiment it is clear, that the strains from *santara* and *malta* inter-cross and pass easily from one into the other. Plate XVI, fig. 3, shows a potted *malta* plant which had been inoculated with a strain *C. gloeosporioides* from *santara*. Two of the branches are dead.

(*j*) *Effect of stocks on scions in producing resistance or otherwise if any*

In the plantations, in the Punjab various grafted plants of *malta* and *santara* are used. Generally the following are used in the Punjab as stocks :—*Khatta, khatti, pomelo, mokri, mitha, kimb, Citrus commerce, turang, Citron pickle* and *Eureka lemon*. In his trial of stocks at Peshawar, Brown [1920] used *khatti, khatta, mitha* and *galgal* as stocks, as these are mostly used by the local nursery men as their stock.

The culture Nos. 345, 346 and 347 were used in most of the inoculation experiments, as these strains were found to be virulent, and inoculated to the *malta* and *santara* leaves. In the following experiments cut twigs from grafted plants were taken and the effect of the stock on the scions on disease resistance or otherwise if any was studied. In one series the twigs were simply sprayed with spore-suspensions in distilled water and in the other before spraying incisions with a lancet needle were made on the twigs near the growing points. *Malta* twigs from grafted plants growing on the following stocks were taken—*Mokri, turang, Citron commerce, khatti, kimb, khatta, mitha, chakotra* and *Eureka lemon*. The experiments were carried out in early April and two twigs of each in each series were inoculated. The twigs were placed in small beakers half full of sterilised tap water and left in a glass chamber with special ventilation arrangement. The leaves of the infected twigs began to fall in four-five days' time. The tip became pale brown to dark brown and in seven-eight days' time acervuli were formed. When tips were infected, there was clear demarcation between healthy and diseased tissue as characteristic of the disease.

Controls were sprayed with distilled water and these remained healthy throughout the period of experiment. On the result of these experiments, it has been found that *Eureka lemon, khatti, turang* and *kimb* as stocks have some effect on the *malta* scions in producing disease resistance to a certain extent.

In the next series of experiments only the cut leaves of *malta* growing on different stocks were inoculated by spraying with spore suspensions after incision in one set and without incision in another. Three leaves were put inside sterilised Petri dishes containing soaked cotton wool. Controls were kept and were sprayed with distilled water, and these remained healthy throughout the period of eight days during which observation was made. It has been found that under very moist conditions and in a bad state of ventilation as inside the covered glass basins—*khatti, turang*, and *kimb* which were found in the previous experiment resistant to some degree, became susceptible. *Eureka lemon* however was found to be resistant to a higher degree.

Extensive field trials could not be done for want of suitable experimental orchards but in a small scale was carried out in the Indian Mildura Fruit Farm Orchard at Renala through the courtesy of the proprietors. Culture No. 346 from *santara* and No. 347 from *malta* were inoculated by means of pricking and spraying on the following varieties of *malta, viz.*, Blood Red, Valentine late, and common *malta* grafted on *kimb, Eureka lemon* and *khatta* stocks respectively. Five plants of each were inoculated in early September, immediately after the rainy season in the Punjab. The plants were under observation for over six months. Though Blood Red has been shown to be a susceptible variety by inoculation experiments, it was found that with *kimb* as stock no infection took place. Similarly Valentine *malta* on *Eureka lemon* remained free from disease. Only the common *malta* on *khatta* showed typical wither-tip.

From the results of the above experiments done in the laboratory and in the field, it may be recommended that *khatti*, *turang*, *kimb* and *Eureka lemon* should be used as stocks when making grafts for *malta* and *santara*.

Conclusion and summary

A major disease of the *Citrus* in the Punjab, *viz.*, wither-tip caused by *Colletotrichum gloeosporioides* Penz. has been studied. It occurs in an epidemic form in many places on *santara* (*C. poonensis*) and *malta* (*C. sinensis*). Though the disease is found throughout the province, it has been noticed that orchards situated near the foot of the hills suffer most. Beside withering of tips, great loss is caused by dropping off of immature fruits. Young plants may be killed outright. The diseased twigs turn silvery grey and become sharply demarcated from the healthy portion. The affected twigs and leaves bear minute dark acervuli around which dark septate or continuous setae are frequently seen.

Studies include growth of the fungus in culture, isolation of different strains and experiments regarding sex in *C. gloeosporioides*, method of infection and penetration, contributory causes, resistance of different species and effect of stock on scions (spraying tests and effect of manuring and aeration are described in the last chapter under control measures).

C. gloeosporioides grows well on various media in culture and the spread of the fungus, spore sizes and various growth characteristics at different temperatures have been given. The effect of concentration of media on growth has also been given. Several strains, showing not only difference in spore-measurements but permanent differences in growth characters, have been isolated and their virulence in causing disease has been tested. Presence of different sex characters in the different monosporous cultures has been found. When two cultures were grown side by side, in some cases complete fusions were seen, in others space of aversion was formed, while others had the white line of aversion. No fruit bodies however have been formed.

Though healthy plants have been successfully inoculated, it has been found that some of the severest injuries in connectior with the disease may be due to the combined effect with other fungi.

Regarding contributory causes, it has been found that under unfavourable soil and climatic conditions or when the trees are injured by hail-storms or by any other means, the causal organism grows vigorously and quickly kills the plants.

With regard to the penetration of the organism into the leaf, it has been shown that the spores on germination in presence of water, give out certain products which react upon the cell walls and as a result the cells below always get injected before actual penetration takes place. Even when a filtered drop of water, in which spores were growing for twenty-four hours at 25°C., was placed on the upper surface of the leaf, injection of the cells below was produced. The infection tube

from the appressoria-like structures produced at the tips of the germ-tubes easily penetrate and enter into the cells below. It has been suggested that nutrient substances diffuse out from the injected cells and stimulate the appressoria to germinate.

For studying the resistance of different species of *Citrus* to the wither-tip, *khatta*, *khatti*, *malta*, *santara*, *galgal*, lemon, and *pomelo* leaves were separately inoculated with two strains of *C. gloeosporioides* which were found to be very virulent. As a result of inoculation experiments, it was found that under very moist conditions infection never failed but under comparatively dry conditions, some of the above plants showed resistance to a certain degree.

Different varieties of *malta* were similarly tested, but no resistant variety was found.

Regarding the effect of stock on scion, it was found that Blood Red *malta* which was proved to be a susceptible variety, was found to be somewhat resistant to wither-tip when grafted on *kimb* as a stock. Similarly the Valentine late or *Eureka lemon* remained free. Only the plants with *khatta* as stocks developed typical wither-tip symptoms. From the results of experiments done in the laboratory and in the field *khatti*, *turang*, *kimb* and *Eureka lemon* have been recommended as suitable stocks for *malta* and *santara* plants.

III. Other diseases of Citrus

(B) Chlorosis

(*a*) *External symptoms*

The leaves show a mottled appearance and drop down early. Plate XIII shows drawings of a few chlorotic leaves of *malta*. Chlorosis is also seen in *santara* but not so commonly as in the other species. When a tree becomes affected, only a few leaves or a few branches may show the symptoms, though in some cases all the leaves may be involved. In the individual leaves, the lighter areas may be confined only to a few spots or almost the whole of the leaf surface may become chlorotic. In the latter case only narrow green bands may be seen along the mid-rib and base of the secondary veins, the whole of the remaining area turning pale green or yellow. The yield of fruits decreases considerably and the fruits become smaller and from badly affected trees, no fruits are obtained. In Valencia, in Spain, Regueral [1932] found considerable reduction of yield from "foliocelosis" (chlorosis) and he mentions general stunting of the branches and elongation of leaves in chlorosis. In Italy, Petri [1926] observed mottling in orchards badly affected with "wither-tip" (*Colletotrichum gloeosporioides* Penz.) but he found no connection between the fungus and chlorosis. Here in the Punjab, chlorosis has been noticed in *malta* plants absolutely free from "wither-tip"; hence there can be no connection with the fungus, chlorosis being really

due to some sort of physiological trouble. Petri [1931] has also descri an infectious mottling of the leaves of *Citrus vulgaris* and, though he has not done any experimental work, he considers from the spreading nature of the disease, that it is carried by insect vectors, *viz.*, *Citrus* aphids which are always present. It may be mentioned here that in the Dhupsari plots of the Lawrence Gardens, Lahore, where the affected *malta* trees have been under constant observation, the *malta* plants are openly laid out and are free from *Citrus* aphids. In this plot plants show chlorosis at random. The chlorosis here is not thought to be of an infectious nature.

(*b*) *Internal structure and chemical nature of the chlorotic regions of the leaves*

Sections of chlorotic and contiguous green portions of the leaves were cut and examined. It was noticed that in the chlorotic portions, the pigments become light coloured and as the chlorotic portions turn yellow and white, the pigments also become gradually separated from the plastids and degenerate and in whiter portions, the plastids, specially of the inner palisade and spongy tissue, degenerate and empty cells may be seen collapsing. Somewhat similar symptoms have been observed by Petri [1931] in what he describes as infective chlorosis. In sections of the chlorotic portion of the leaves, it is a common thing to find the light green pigments which break down forming minute granules, to be seen in a state of motion.

Regarding chemical reaction of the chlorotic portions, Petri [1926] found that an excess of acid accumulates in the chlorotic region as compared with a contiguous green area. Sometimes it has been suggested that deficiency of calcium in the soil produces this condition. Regueral [1932] recommends application of calcium to stop this malady. But it has been shown that no correlation could be found between soil acidity or alkalinity and chlorosis. In Lahore, the soil from below the trees in the observed plants was tested, but no lack of calcium was noted, the pH value being 8·8. The soil from below the trees in different places showing chlorosis was also similarly tested and found to vary from 8·2 to 9·0 pH. In no case any dearth of calcium was noted. The pH value of the normal green and contiguous chlorotic portions of the leaves has also been tested. By means of a fine pair of scissors the different areas were cut out and the sap from the normal green and chlorotic areas were extracted separately by pressing in a vice between two thick ebony plates. As noted by Petri [1926] the chlorotic portions showed greater acidity. A large number of extractions was tested and the pH value of the chlorotic sap was found to lie between 6·4 and 6·6 and those of the normal green portions between 7·0 and 7·2. It may be mentioned that the pH was tested in early May when the leaves were comparatively young, and perhaps more acid might accumulate later when the leaves became older. This will again be done, but no correlation between accumulation of acid in the chlorotic regions and lack or excess of calcium in the soil could be made out in any case.

(c) *Experiments on the control of chlorosis of* malta

The injections of chemicals into plants for the control of fungous and bacterial diseases have been tried by various workers from time to time, but the results have been conflicting. This is apparently due to the fact that a solution of any chemical that will injure the protoplasm of the causal organism will also injure the protoplasm of the host-plant cells. Recently the injection of iron salts into the trunks of trees for combating chlorosis of various plants has received considerable attention. Bennet [1927] effected a ninety per cent cure of chlorosis of pear trees by injection of iron salts, and Wann [1930] by placing dry salts of ferric ammonium in holes bored into the stem of grape vines, peach and apple trees effected a similar cure ; the best response, however, was obtained by him from ferric phosphate. Thomas and Haas [1928] checked chlorosis of orange trees by injection of iron salts but they found subsequent injections necessary, since the wood was injured for some distance along the point of injection. A short note on the curative value of iron salts when injected has been published by the author [Chaudhuri, 1933]. In the experiments for the cure of the chlorotic condition of the *malta* leaves carried out here, the efficacy of different iron salts has only been tried. These salts have not only been injected but also sprayed over the plants with beneficial results. The experiments were carried out in early November with chlorotic *malta* plants only. In the preliminary experiment, cut twigs of *malta* bearing chlorotic leaves were placed in 0·01, 0·001 and 0·0001 per cent of the following salts : Ferric phosphate, ferrous phosphate, ferric sulphate, ferrous sulphate and iron-alum. Control twigs were placed in tap water. These were kept under observation for seven days in a big glass chamber, the atmosphere being naturally much more humid than that outside. It was observed that in solutions 0·01 and 0·001 per cent all the twigs except those in iron-alum became dead in three days. The leaves of the chlorotic control twigs also dropped down and in four-five days, the leaves of the non-chlorotic control twigs also dropped down. In iron-alum, 0·01 per cent solution, it was observed that the twigs remained very fresh for over seven days but no recovery from chlorosis was noticed and in dilute solutions of iron-alum, the leaves dropped down in three-four days. The other iron salts, however, proved beneficial in very dilute solutions (0·0001 per cent), and partial recovery from chlorosis was specially noticed in ferrous sulphate and ferrous phosphate solutions.

In the second series of experiments, cut twigs with chlorotic leaves were first placed in normal culture solutions. After twenty-four hours these were removed and placed in 0·0001 per cent of the different ferric and ferrous salts for twelve hours and then were replaced in the culture solution. Similar results were observed. The efficacy of the salt solutions in 0·0001 per cent solutions may be put in the following order :—

1. Ferrous sulphate—most helpful, many recoveries.
2. Ferrous phosphate—helpful, partial recovery.
3. Ferric phosphate and ferric sulphate—very slight effect, if any.
4. Iron-alum—no recovery.

Simultaneously with the above experiment, in another set detached chlorotic *malta* leaves were placed in large glass basins with cover over soaked cotton wool and sprayed with 0·0001 per cent solutions of the above salts. Partial recovery was noticed only with ferrous sulphate and ferrous phosphate solutions. Hence these two were selected for field experiments.

Solutions of these salts, 0·0001 per cent, were made and 2·3 c.c. were introduced very slowly at a time by means of a hypodermic syringe. Two injections were made in each twig. After three days the twigs injected with ferrous sulphate solution showed definite recovery. Simultaneously, when some twigs were injected, other chlorotic plants were profusely sprayed with ferrous sulphate and also with ferrous phosphate solutions ; these showed recovery. The new leaves that developed in plants sprayed with ferrous sulphate showed freedom from mottling, though in the following year the plants again formed chlorotic leaves. There can, however, be no doubt that ferrous sulphate in 0·0001 per cent solution helps recovery from chlorotic conditior and as injection method requires special care and technical skill, spraying with ferrous sulphate is recommended and spraying twice in the year will no doubt be found useful in curing chlorotic condition of *malta* trees.

Summary

Chlorosis of *malta* (*Citrus sinensis* Grove) has been investigated. Chlorotic areas become slightly more acid than the adjoining green portions. No correlation with the soil condition has been found. In transverse sections the chlorotic areas show light green chloroplastids and in bad cases where the regions turn yellow or whitish, break-down of the granules is seen and also the plastids degenerate and the cells become empty and collapse. Injections and spraying with different iron salts have been tried for curing chlorotic condition. Ferrous sulphate in 0·0001 per cent solution, whether injected or sprayed, has been found to be most efficacious and as spraying requires no special skill, this method has been recommended.

(C) Saprophytic moulds

(*a*) *Symptoms*

These are found to a certain extent in almost every garden, but in certain orchards they appear in a severe form and practically cover all the leaves (Plate XIV) and even fruits with a black incrustation, consisting of a weft of black mycelial threads of fungi of more than one species usually. When complete incrustations are formed, the sunlight is to a great extent cut off and consequently normal physiological processes of the leaves are interfered with. Not only does less formation of starches and sugars so necessary to the growth of the plant take place, but it has been observed that the fruits so covered have a sour and fermented

taste. Often the yield becomes very much reduced. Where fruits are covered in patches by the sooty mould incrustations, they develop a mottled appearance which is seen on cleaning the fruits. This is due to the fact that the covered patches do not ripen with the rest of the fruit surface and there retain a light green colour. Sometimes leaves covered with sooty mould curl and shrivel up under dry conditions. The black covering of sooty mould is, however, entirely superficial and may easily be rubbed off. Under dry conditions, they themselves become detached and are blown off in bits by the wind. They do not enter into the tissues of the leaves, but live on the honey-dew secreted by the scale insects in the wake of which they follow, and the control of the disease depends to a great extent on the control of the insects, for without their excretions, they will soon disappear.

Abundance of sooty moulds is generally found in the orchards where the trees are very closely planted or where the trees are shaded by other tall trees around them, so that light is interfered with and the atmosphere remains more humid. The following fungi have been found to take part in forming the sooty incrustations, *viz.*, *Acrothecium lunatum*, *Capnodium citri*, *Alternaria citri*, *Cladosporium herbarum* var. *citricola* and *Chaetomium* sp. The ascigeral fungus, *Pleospora herbarum*, has also been isolated. Any one of the first four species may predominate. Composition differs in different places, perhaps also in different seasons. The presence of *Alternaria* in sooty moulds may lead to infection of the fruits at the stylar end causing black rot of oranges and also dropping of immature fruits. *Acrothecium* may also produce a similar black rot of the fruits if it can gain entrance.

(*b*) *Acrothecium lunatum* Wak.

It has been isolated from the black incrustation on the leaves and grown in culture on various media. The conidia are curved, knee-shaped, 2-4-septate with large middle curved cell (Plate XVIII, fig. 2). It measures $18{\cdot}22 \times 5{\cdot}7\ \mu$. It grows well on a large variety of media. On starchy media like potato agar, pea agar, bean agar, etc., greater development of aerial hyphæ takes place but the spores are usually smaller, whereas, in synthetic media like Raulin's, etc., lesser development of aerial hyphæ takes place but the spores are bigger. It has been grown on various concentration of Raulin's. In higher concentrations, no spore formation took place and the mycelium developed a slight pink colour and in very low concentrations ($N/32$), scanty development of hyphæ took place and the growth was zonal. Regarding width of the spores it was found that the mean breadth from N to $N/32$ concentration decreased gradually from $6{\cdot}9\mu$ to $5{\cdot}2\mu$ and the area of spores (length × breadth) also uniformly decreased from normal to $N/32$ concentrations, though the greatest mean length of the spores was found at $N/16$. In hanging drops, the spores germinate readily. Germ-tubes are ormed either from one end or both ends. Inoculation experiments have also

been done. Pug inoculations were made. The inoculum was introduced inside both *malta* and *santara* fruits. In a week's time when the fruits were cut, jet black rot was seen. The fungus was re-isolated from the inoculated fruits. Control lies in removing the source of contamination by spraying the leaves with fungicides.

(*c*) *Capnodium citri* Berk. et Desm.

This fungus (Plate XVIII, figs. 1-2) lives entirely superficially on the leaves and fruits of *Citrus* sp. on the excretions left by the scale insects. In *Citrus*, it never gets inside the tissues and causes no rot. In certain regions and in certain seasons, this forms the predominating sooty mould fungus. Fawcett and Lee [1926] mention that "when it occurs in abundance, it may seriously retard the growth, causing light blooming and flowering, and rendering the tree less resistant to drought." The curling and shrivelling of leaves affected by sooty mould under dry conditions has already been mentioned. The colour of the hyphæ varies from olive-green to deep brown, and they form a dark brown crust on the surface. This fungus reproduces by conidia, pycnidia, stylospores and perithecia, and these have been described and drawn in detail by Webber [1897]. The measurement of the different kinds of spores varies a lot and they grow readily in cultures. Conidia, pycnidia and stylospores are abundantly formed in cultures, but their shapes and sizes differ very much. Stellate spores of *Triposporium* form are readily formed from the hyphæ (Plate XVIII, fig. 2). These germinate readily. Control by spraying with fungicides.

(*d*) *Alternaria citri* Pierce

It is almost always associated with the sooty mould fungi and may cause dropping of fruits and also a black dry rot of the fruits if it gets inside the fruits. In many parts of the Punjab, *Alternaria* rot is common. It gets in either through the stylar end or stem end of the fruits which gradually becomes pale and discoloured. *Alternaria* can only get in through some injured tissue produced either mechanically or through insects. The rot is seen when the fruit is cut open. *Alternaria citri** has been isolated both from the sooty-mould incrustations on the leaves and fruits and also from the rot inside the fruit. The culture from the mould as well as from the fruit rot have been grown side by side in various media and have been found to be identical. This fungus grows readily in almost any media and the spores which are septate are in chains (Plate XVIII, fig. 4). These spores also germinate readily, and the germ-tubes may be formed from the two end cells or even from each cell of the spore. The measurement of the

*As *Alternaria* spores are almost always present, care should be taken when picking the fruits, otherwise through the least injury, the fungus will get in and produce rot in storage.

spores in various media shows that they differ a good deal. Effect of different concentrations of Richards' medium on growth and spore formation was studied. *Alternaria* grew very well at 8*N* concentration when the largest size spores were also formed. Inoculation experiments have been made and it has been found that it is a wound parasite. Plug inoculation of fruits produced the characteristic rot. As the source of contamination is always present in the sooty moulds, spraying with fungicides to remove them will prove effective in checking it.

(*e*) *Cladosporium herbarum* var. *citricola* F. & B.

It is very commonly found in the sooty-mould incrustations of the *Citrus* leaves. This fungus also causes what is known as "leprosis" or "nail head rust" of sweet oranges. It produces sunken spots on the rind. The fungus readily grows in culture and abundant spores are formed (Plate XVIII, fig. 5). The spores vary in shape and size (8-12·6μ × 2·7-3·1μ). In culture, the fungus develops a greenish tinge.

(*f*) *Chaetomium* sp.

Sometimes it forms an important fungus in the socty-mould incrustations. It is a purely superficial fungus like the *Capnodium* living on the excretions of the scale-insects. It forms dark black hyphæ and perithecia with asci are often seen and are formed readily in cultures (Plate XVIII, fig. 7). It may readily be removed by spray mixtures.

(*g*) *Pleospora herbarum* Rab.

It may be looked upon as quite a common black mould of *Citrus* leaves in many parts. Perithecia formed on almost any media—even on moist cotton wool plenty of fruit bodies are formed. Ascospores germinate readily (Plate XVIII, fig. 6). Other species of *Pleospora* are found on dead twigs of *Citrus* forming fructifications.

Conclusion and summary

Organisms taking part in the formation of sooty-mould incrustations of *Citrus* leaves and fruits in the Punjab have been isolated and their growth in culture studied. Though not much actual damage is done to the leaves by the sooty coverings, the fruits so covered turn bitter in taste. Besides some of the organisms taking part may cause important diseases and always remain as a perpetual source of infection unless properly controlled. For instance, *Alternaria citri* may cause considerable loss in conjunction with *C. gloeosporioides* by bringing about fall of immature fruits. Similarly *Cladosporium herbarum* var. *citricola* may cause leprosis or nail head rust of sweet oranges.

Six different fungi, *viz.*, *Acrothecium lunatum*, *Capnodium citri*, *Cladosporium herbarum* var. *citricola*, *Alternaria citri*, *Chaetomium* sp., and *Pleospora herbarum* have been commonly found to take part in the formation of sooty incrustations of *Citrus*. Most of these are known to occur on *Citrus* and other fruit trees in different countries. Preti [1923], Janini [1923], Tengwall [1924], Bolle [1924], Fraser [1933-1934] and others have investigated the composition, systematy and physiology of the fungi. Beside the forms noted above *Aspergillus* is also readily found in the sooty incrustations. All those moulds which follow in the wake of the scale-insects and live on their excretions, may be effectively controlled by controlling the scale-insects. Details regarding spraying for their control have been given in the chapter on control measures.

IV. Control measures

(*a*) *Control experiments at Lahore.*

Extensive experiments for the control of the disease in the field by means of spraying, manuring, aerating, etc., have been done, and will be described later in this chapter. First a few experiments to see the efficacy of certain spray mixtures in preventing the disease are described. In the preliminary experiments done in the laboratory only Bordeaux mixture (5 : 5 : 50) and two proprietory mixtures, *viz.*, Sulfinette and Arboretas Wash were used. The experimental plants were kept under observation for over two months and only disease-free plants were selected. These were first sprayed with the fungicides and were then sprayed with spore suspensions after a week.* After a fortnight all the *malta* and *santara* plants excepting those previously sprayed with Bordeaux showed positive infection. Plants sprayed with Sulfinette and Arboretas Wash, though infected, were no doubt in a much better condition than the unsprayed plants which were very badly infected.

Systematic spraying of fungicides for the control of wither-tip and other associated diseases was taken up from early November, 1931. At that time the plants were all bearing fruits and hence no pruning could be done. A very badly affected plot was selected in a garden (Mangal Singh's garden, near Shahdara) near Lahore. Almost all the twigs of the plants showed wither-tip (Plate XV) and every day a large number of immature fruits were dropping on account of infection by *Colletotrichum* and sometimes also on account of other organism. The affected fruits showed pale yellow colour, and from a large number of fruits the

* For the preparation of fungicides the following books have been freely consulted :—

Anderson and Roth	.	Insecticides, fungicides and Appliances, 1923.
Bourcart and Burton	.	Insecticides, fungicides and Weed Killers, 1926.
Mason . .	.	Spraying and Dusting and Fumigating of Plants, 1929.
Fawcett and Lee .	.	Citrus Diseases—Chapter on fungicides, etc., 1926.

causal organism was isolated. These fruits also become bitter in taste and rather sour. In this plot, the leaves of the plants were all covered by sooty moulds. Two different spray mixtures were tried, *viz.*, Bordeaux Paraffin oil emulsion and Sodium sulphate-kerosine oil emulsion. The following shows the result of spraying :—

Date of spraying	No. of trees sprayed	Spray mixture	Quantity sprayed	Remarks
2nd Nov. 1931	8 *mitha* . 1 *galgal* .	Sodium sulphate kerosine oil emulsion. Sodium sulphate 6 oz. Kerosine oil emulsion 650 c. c in 12 gall. water.	12 gall. .	Not very effective for fruit-fall but prevents sooty-mould.
4th Nov. 1931	30 *malta* .	Bordeaux Paraffin oil emulsion. Copper sulphate 4 lbs. Unslaked lime 4 lbs. Paraffin oil emulsion 1 gall. Water 49 gallons.	40 gall. .	Spray mixture spreads uniformly and sticks for a long period. After a fortnight fruit-fall checked. No further spread of disease.*

Four *mitha* and four *malta* were left untreated as control plants. In these the fruit-fall continued unchecked.

The Dupsari Citrus plot in Lawrence Garden, Lahore, where the plants were badly attacked by wither-tip and other diseases, was selected for testing the efficacy of the different spray mixtures, *viz.*, Bordeaux mixtures (4 : 4 : 50 and 5 : 5 : 50), Bordeaux with crude oil emulsion, Ammoniacal copper sulphate, Home-made lime sulphur and also the following proprietory fungicides, *viz.*, Arboretas Wash, Cooper's fungicide, and Sulfinette. As this plot was badly, infected, there were very few fruits on the trees. These trees were heavily pruned before first spraying was done. All cut twigs were carefully collected and burnt. The second spraying was done with the same spray mixtures on the same trees in middle of May, after new fruits had been formed. The spraying was always done in the afternoon. The details regarding spraying and the results are shown in Table IX.

* In the following March these plants were pruned heavily and sprayed once more before the flowers came out. Next season there was practically no loss from fruit-fall in that plot.

TABLE IX

Result of spraying of malta *trees with different fungicides. First spray as on date in the table, second one during middle of May 1932.*

Date of spraying	No. of trees	Spray mixture	Quantity sprayed	Remarks
			(Gallons)	
2nd Dec. 1931 .	12	Bordeaux (4 : 4 : 50).	10	No spray injury, many twigs dying back, on dead twigs fruiting bodies of *C. gloeosporioides* and also of *Gloeosporium* appear, leaves pale green in colour, fruits bigger.
3rd Dec. 1931 .	14	Sulfinette (1 in 40).	8	Young leaves were scorched and later all twigs and branches showed wither-tip progressing.
4th Dec. 1931 .	21	Cooper's fungicide (1 in 16).	10	These plants had no fruits, no spray injury but anthracnose spots appeared on leaves, later, twigs showed wither-tip.
10th Feb. 1932	52	Cooper's fungicide (1 in 16).	22	Results as above.
17th Feb. 1932 .	28	Sulfinette . .	12	Scorching seen, fruits sunburnt, twigs also showed wither-tip progressing.
Do .	38	Bordeaux(4 : 4 :50)	20	No injury, but disease not checked.
Do. .	18	Bordeaux(5 : 5 : 50)	10	No injury, better result than Bordeaux (4 : 4 : 50) some plants (5 out of 18) badly affected.
Do. .	15	Bordeaux and crude oil emulsion.	6	No injury, trees healthier, 5 out of 15 plants showed mild wither-tip.
Do. .	24	Ammoniacal copper carbonate.	17	No scorching and injury to fruits, disease held in check for some time, but later twigs showed wi- ther-tip.
Do. .	38	Home-made lime sulphur.	13	No injury, no scorching of fruits, similar in effect to Bordeaux (4 : 4 : 50).
Do. .	80	Arboretas wash .	7	The wash is perfectly safe to use, young leaves not scorched, trees have healthier appearance, disease checked, 22 *malta* out of 80 showed disease.

Half a dozen *malta* trees showing wither-tip were left untreated as control plants during the progress of the experiment. At the end of two years, these plants showed wither-tip right up to the stump—the upper portions being dead. These were then cut down and burnt. Regarding the efficacy of the different spray mixtures as seen from the above experiments it may be summed up that Bordeaux-oil emulsion and Bordeaux mixture (5 : 5 : 50) proved helpful and of the proprietory fungicides Arboretas Wash proved a useful one. The cost of spraying per tree per spray is given below :—

	Rs.	A.	P.
Ammoniacal copper carbonate	0	2	9
Arboretas Wash	0	1	0
Bordeaux-oil emulsion	0	0	9
Sulfinette	0	0	9
Home-made lime sulphur	0	0	5
Cooper's fungicide	0	0	3
Bordeaux mixture	0	0	3

In July 1932, the orchard adjoining the Superintendent's Bungalow, Lawrence Garden, Lahore, was chosen for spraying with fungicides. This plot contained mostly *malta* plants and all the plants, without exception, were affected by wither-tip. Three hundred and thirty plants were sprayed with 325 gallons of Bordeaux-crude oil emulsion. The cost per tree for spraying worked at nine pies per tree (including labour) as follows :—

	Rs.	A.	P.
Copper sulphate—16¼ seers	8	2	0
Lime—16¼ seers	0	8	0
Crude oil—1½ gall.	1	14	0
Soap—1 seer	0	8	0
Other charges (carrying spray machines, vats, etc.)	2	6	0
Total	13	6	0

At the time of spraying in July a large number of fruits were daily dropping down on account of the stem-end-rot caused by *C. gloeosporioides*. On dissecting the fruits the dry rot could be seen progressing through placenta into the pulp. Sometimes the fruits dried up and remained attached to the stalks. The stalks from the bottom end (2-in. to 3-in.) sometimes dried up and acervuli were seen developing on them.

As a result of spraying, further fruit-fall was checked, but the result on the whole was not as satisfactory as might be expected, for during the weeks following

spraying, there were frequent showers, which washed off much of the spray materials. Hence later on Bordeaux mixture with either ferrous sulphate as a sticker * or Rosin † for the same purpose was used. Rosin sticker was used in plots where plants suffered from sooty mould as well. Sometimes Bordeaux-rosin sticker with nicotine sulphate ‡ specially where scale-insects were present, were used. The use of the last-named spray mixture, however, is objected to by the Sikhs on religious grounds as it contains nicotine. So Bordeaux-rosin which is a cheap and effective fungicide is recommended for use. In many of the orchards chlorosis is a common form of ailment, and in those cases Bordeaux with a ferrous sulphate sticker has proved of great help (chlorosis and its control have been dealt with separately in another chapter).

In a private garden at Lahore seven *Citrus* (four *malta* and three *santara*) trees were very badly affected by wither-tip and bore branches which were killed 2 ft.-3 ft. downwards. The trees were almost dying. Leaves showed mottling. These were heavily pruned and sprayed with Bordeaux containing ferrous sulphate as a sticker (22/1/37). There was no injury to the leaves, and though it rained several times, the fungicide was not washed off (6/3). This fungicide proved very effective. The plants all revived, grew well, no wither-tip appeared and the leaves were no longer mottled but developed a healthy green colour. Next season, all these plants fruited very well.

In the laboratory, forty other *Citrus* plants (*malta*, *santara* and *mitha*) which were flowering at that time (7/3) and were full of scale insects, were sprayed with Bordeaux (3 :3 : 50) with rosin-sticker. When examined after a fortnight the plants were found free from the aphids, and it was seen that there was practically no injury done to the flowers. In another lot of plants growing in pots and attacked by scale-insects, spraying was done with Bordeaux with Rosin-sticker and nicotine sulphate with very good results. To 12½ gallons of Bordeaux mixture (4 : 4 :50) 60 c.c. of nicotine sulphate (40 per cent) and 1,000 c.c. Rosin-sticker were added. This spray may be used in plants in which the fruits have set with very good result, but should not be used in flowering time. In the laboratory, six plants which were flowering at the time of spraying did not bear any fruits.

In July 1933, three hundred *Citrus* plants (mostly *malta*) in the Dupsari plot were sprayed with 350 gallons of Bordeaux mixture (5 : 5 : 50) with Rosin-sticker after pruning. These plants had been sprayed in the previous season. Three hundred and thirty plants in the orchard adjoining Superintenent's Bungalow, which had in the previous season been sprayed with Bordeaux mixture (5 : 5 : 50), were sprayed with 350 gallons of Bordeaux with ferrous sulphate as a sticker.

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* Copper sulphate 5 lbs., ferrous sulphate 4 lbs., lime 10 lbs., water 50 gallons.

† Rosin 2 lbs., Washing soda 1 lb., Water and Bordeaux mixture 50 gallons. (4 : 4 : 50).

‡ With Bordeaux-rosin (50 gall.), ½ pint of Nicotine sulphate (40 per cent) was used.

Both the above mixtures have proved very useful but the errous sulphate sticker sticks for a much longer time. Leaves of plants sprayed before the rainy season (in May) bore traces of the spray long after the rains (October). The former, *viz.*, the Bordeaux with Rosin-sticker costs seven pies per plant per spray and the latter six pies per plant per spray.

The plot adjoining the Superintendent's Bungalow was once more sprayed in February 1934, and this plot with over 300 plants may be said to be free from the disease. The yield of fruit last season was very high and there was practically no loss from fruit-fall.

(*b*) *Control experiments at Nurpur*

Nurpur is situated at the foot of the hills in the Kangra Valley. It is a very important centre for *Citrus* cultivation and in and around Nurpur, there are quite a number of large *Citrus* plantations. Sooty moulds heavily cover the leaves of most of the trees in this area and wither-tip disease has appeared in rather an epidemic form.

Through the courtesy of the Hon'ble Mr. Justice Tek Chand his *Citrus* orchard just opposite the Nurpur Railway Station was placed at our disposal for control experiments. Bordeaux mixture with Rosin-sticker was exclusively used. Sixteen hundred plants (*santara* 1,277, *malta* 150, *nimbu* 113 and *galgal* 60) were sprayed with 1,600 gallons of the spray at a cost of seven pies per tree (1 : 6 : 33), after these had been lightly pruned. The spray killed the sooty moulds which fell off in crusts*, and though rains started after a fortnight the spray was not completely washed off (29 : 8 : 37). The plants improved very much in appearance, and there was practically no loss from fruit-fall. When in the following February, the plants were heavily pruned and sprayed once more with the same fungicide there was very little of wither-tip or sooty mould. The plants fruited more plentifully. Most of the growers in that area took much interest in our work, and as they were convinced of the usefulness of these fungicides, many of them have taken up control measures according to our directions.

(*c*) *Recommendations for disease control by spraying of fungicides*

For the control of the wither-tip and the associated diseases, Bordeaux mixture (5 : 5 : 50) with ferrous sulphate as sticker, where sooty moulds are absent, or with Rosin as a sticker, where sooty moulds are present is strongly recommended for use. As the sticking power of ferrous sulphate is greater and as it checks mottling to a great extent, its use as an alternative spray mixture after using Rosin-sticker to remove and control the scale-insects is recommended. For plants which are badly attacked by the aphids, on the excretions of which the scale insects live, nicotine sulphate with Bordeaux-rosin may be used as a first spray, but it should never be used when plants are flowering.

* When these crusts were plated, the spores did not germinate.

In this connection a few words about quarantine treatment of imported plants for the prevention of disease will not be out of place. In India many nurserymen and private owners import *Citrus* plants and stocks from Australia and other places. At the Bombay Custom House, the plants are fumigated by hydrocyanic acid gas. To find out the efficacy of the treatment a packet containing *Citrus* twigs badly infected by wither-tip and *Citrus* canker, was made and sent to the Chemist at the Custom House at Bombay for fumigation. The packet was loosely wrapped in muslin. It was treated to three hours' exposure of about 0·07 grm. per cubic foot of hydrocyanic acid gas to start with. The concentration is only approximate, for as the Custom Chemist says, it is arrived at by calculation on the basis of the quantity of potassium cyanide used to generate hydrocyanic acid gas and the cubical contents of the fumigation chamber.

When the packet was received back in Lahore, thick spore suspension was made from the spores in acervuli and several platings on potato-agar were made and incubated at 25°C. After three days the plates were absolutely full of colonies of *C. gloeosporioides*. Plating of the canker organism from the treated material also showed that they were not in the least affected by three hours' treatment of hydrocyanic acid gas at that strength, and the number of colonies formed in both cases was similar to those from the untreated ones which were made for comparison.

(*d*) *Control of disease by manuring and aeration*

It has many a time been claimed that plants resist disease if they grow under a healthy condition inducing vigorous growth. In *Citrus* plants, in the Bombay Presidency, the die-back disease has been claimed to be successfully controlled by proper manuring and aeration [Sahasrabuddhe, 1927 ; Cheema and Bhatt, 1929]. To find out if vigorous growth could help the *Citrus* plants in checking the wither-tip disease, experiments on manuring and aeration and also the combined effect of the two have been done both in Lahore and in Nurpur ;

Only two kinds of manures, *viz.*, sulphate of ammonia and Niciphos Grade II (containing equal quantities of nitrogen and phosphoric acid, *viz.*, eighteen per cent nitrogen and eighteen per cent phosphoric acid) supplied by the Imperial Chemical Industries were tried. For applying the manure the soil is watered first and then a 6-in. to 9-in. deep groove is cut round the trees where the spread of the branch finishes. About two lbs. of manure is now put in the trenches round each tree and thoroughly mixed with the soil.

In Dupsari plot in the Lawrence Garden, Lahore, forty *malta* trees were treated with Niciphos and forty-four with sulphate of ammonia, twenty were untreated and kept as control and in the plot adjoining the Superintendent's Bungalow forty *malta* were treated with Niciphos and sixty with sulphate of ammonia, but in between the rows of plants (distance approximately 15 ft.) trenches two ft. wide and three ft. deep were dug. The trenches were cut a month previous to manuring the plants. Between these rows of untreated plants two similar trenches were

dug and two rows of plants were left untreated and without any trenches by their side. The trenches were dug at the end of July. It rained off and on throughout August and September and after three months, *i.e.*, by the end of October the trenches were filled up. In Nurpur Control Station, experiments with manuring only were made, but no trenches were dug. Manuring was done at the end of August. Thirty-two *santara* and twenty-six *malta* plants were treated with Niciphos and thirty-two *santara*, fifteen *malta* and thirty-two *galgal* plants were treated with sulphate of ammonia.

The cost of treating each plant with Niciphos was Rs. 0-3-3, and with sulphate of ammonia Rs. 0-2-2 only. The experimental plots in Lahore were visited every fortnight and the Nurpur Station every two months. It may be mentioned at the outset that no appreciable difference in the growth and vigour of plants was found by aeration only. Perhaps aeration might improve the condition of the plants in ill-drained soils. But manured plants showed distinct superiority over the unmanured ones, the newer leaves being of a deeper green colour, and there was greater abundance and bigger size of fruits the following season and in this respect Niciphos proved a better manure. As most of these plants had been under control spraying, the check of wither-tip in these plants could not be ascribed to manuring only. But a few plants which had not been sprayed but treated with manure, though improved very much in general condition, showed the presence of wither-tip as before. That manuring alone cannot prevent the disease is evident from the experiments carried out by the Imperial Chemical Industries themselves in the Government Botanical Garden, Saharanpur, where a new orchard treated regularly with different kinds of manures from the beginning, showed the presence of wither-tip and canker in many of the plants.

(*e*) *General observations*

To prevent the disease and to deal effectively with it when it has appeared, the orchardist must properly nourish his plants, prune the diseased twigs every season and burn those twigs and as a routine work spray once immediately after pruning (February-March) and once more late in September or early in October when the fruits are growing, according to the directions given already. Not only have the orchards to be maintained in a disease-free condition, but it is equally or perhaps more important to keep the nurseries in a sanitary condition for, as mentioned already, the diseases in the Punjab have in recent years been found to spread mostly from the nurseries which are very badly infected by wither-tip and canker. Owing to ignorance, people buy infected plants. For this, dissemination of knowledge about diseases in plants is essential. The author [Chaudhuri, 1936] published a scheme for this purpose. Growers should be advised on simple control methods, and nursery plants have to be sprayed regularly for protection against diseases ; also these have to be treated for disease if any appears. There should be arrangement for regular inspection of nurseries and granting of certi-

fication of health for nursery stocks by a competent authority and the certificate should be produced at the time of sale of plants. The public should be advised not to buy any plants from an uncertified nursery. For certification work, the teachers of plant pathology in the Universities could easily co-operate with the Government Agricultural departments. Thus not only the wither-tip disease of the *Citrus* but many other diseases could be effectively controlled.

Mention has already been made, in a previous chapter, about the encouraging results obtained regarding the effect of stock on scion in resisting wither-tip disease of *Citrus*.

SUMMARY

Spraying tests, including trials of proprietory mixtures and the effect of manuring and aeration, are described.

Systematic spraying with the following fungicides for the control of wither-tip and other associated diseases was done in selected orchards in Lahore and Nurpur :—Bordeaux and Burgundy mixtures, Ammoniacal copper carbonate, Home-made lime sulphur, Sulfinette, Cooper's fungicide, Arboretas Wash, etc.

Bordeaux mixtures proved most effective but as it may often be washed away during rains, Bordeaux with ferrous sulphate as a sticker has been successfully used and it also helps to reduce chlorosis of the leaves found in many orchards. In many orchards sooty moulds cause trouble in addition to wither-tip and other diseases. Experiments with combined spray mixtures have given satisfactory results. Bordeaux-oil emulsion, Bordeaux with Rosin and Bordeaux-rosin with nicotine sulphate have given excellent result.

Spray mixtures should not only be cheap and easy to prepare but also must not contain ingredients which may be objectionable on religious grounds. Manuring and aerating improved the general conditions of the plants but did not prevent the disease.

Nursery attacks are very serious and control has been discussed under the following heads :—propaganda in nursery sanitation ; need for mycological control, certification of health for nursery stock and advice to growers on simple control methods.

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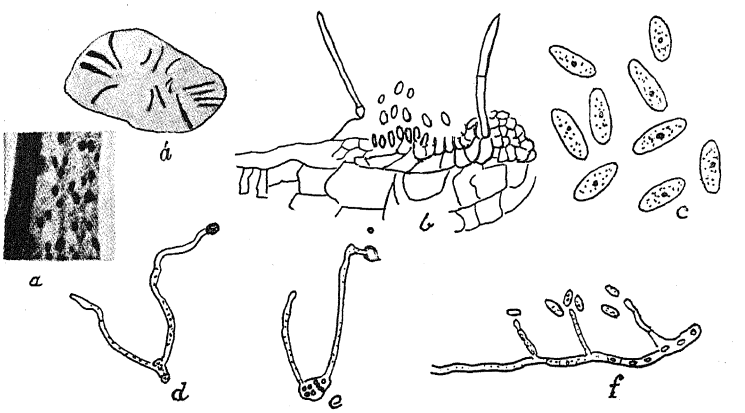


(*a*) Photograph of a twig of *malta* showing acervuli of *C. gloeosporioides* (slightly magnified). (*á*) acervulus with dark setae (surface view) (highly magnified). (*b*) Section through an acervulus (greatly magnified). (*c*) Spores (×650). (*d*), (*e*) Germination of spores. Note dark coloured appressoria produced (×350). (*f*) Spores formed in a hanging drop culture (×350).

A twig of *malta* showing *chlorosis* of leaves ($\times\frac{1}{2}$).
Note also wither-tip.

Sooty mould of *Citrus* leaves. ($\times\frac{3}{4}$).

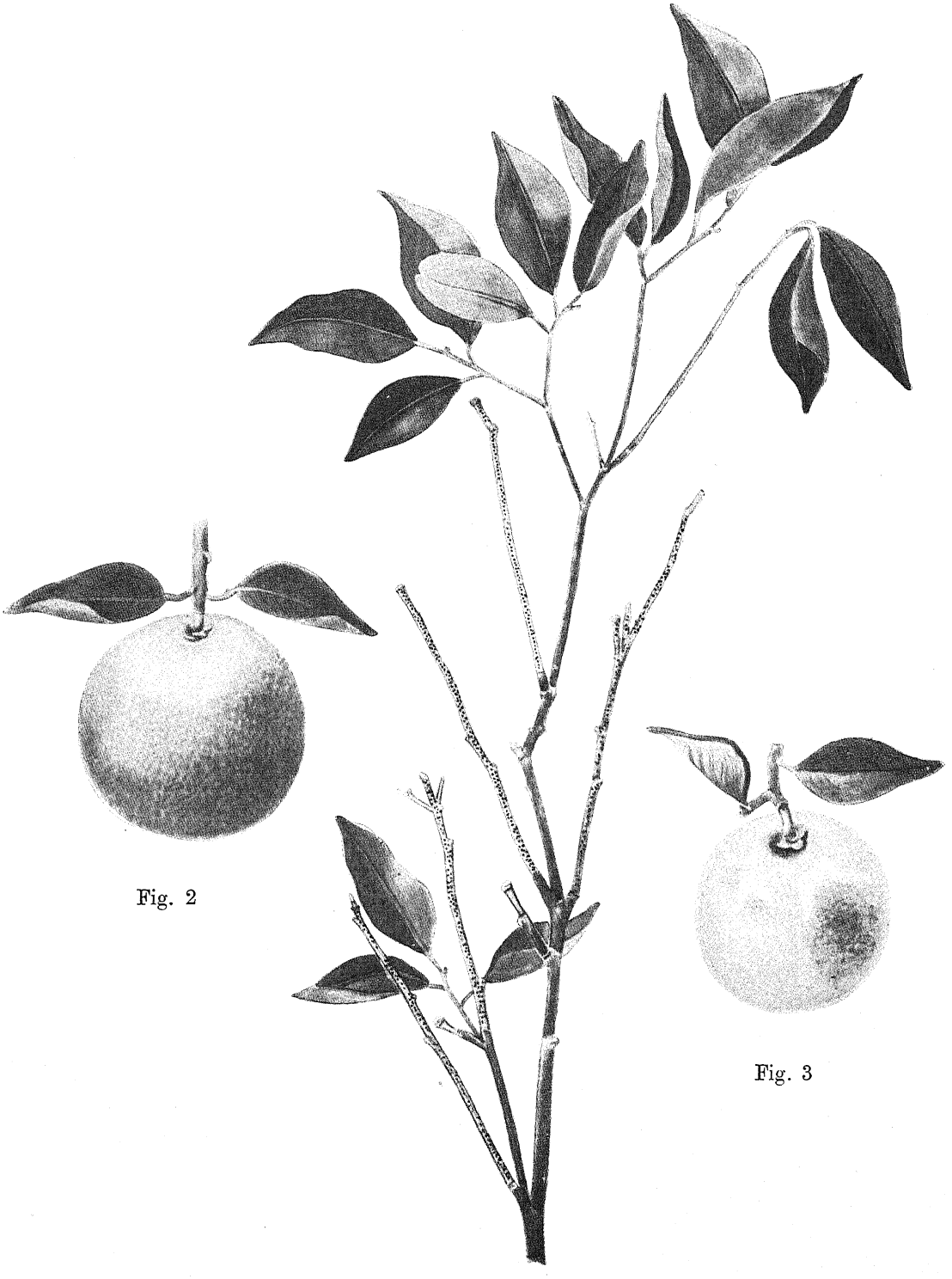


Fig. 2

Fig. 3

Fig. 1

FIG. 1. Twig of *malta* showing wither-tip of all the branches and stem-ends of the fruits.
FIGS. 2 and 3 show stages in dropping of the fruits owing to stem-ends being attacked by *C. gloeosporioides*. ($\times\frac{1}{2}$).

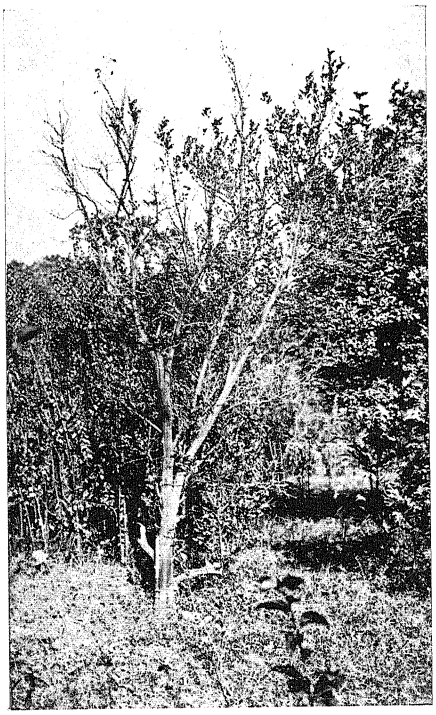


FIG. 1. A *malta* tree badly infected by *C. gloeosporioides*. Most of the twigs and branches are dead. (×1/40.)

FIG. 3. A *malta* plant artificially inoculated. Note two of the branches are dead. (×1/16.)

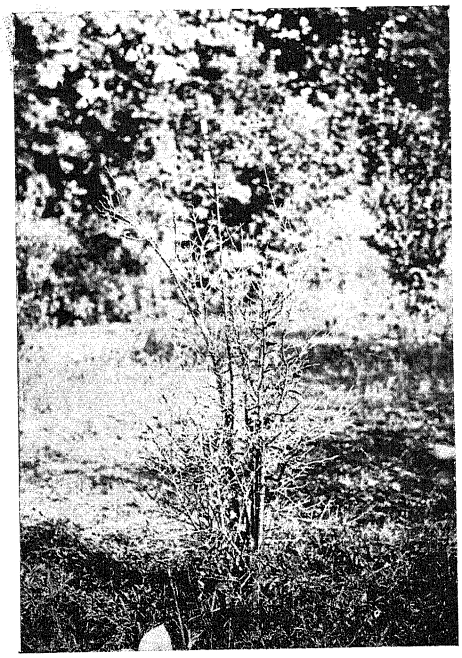


FIG. 2 A *santra* tree almost dead owing to attack of *C. gloeosporioides*.

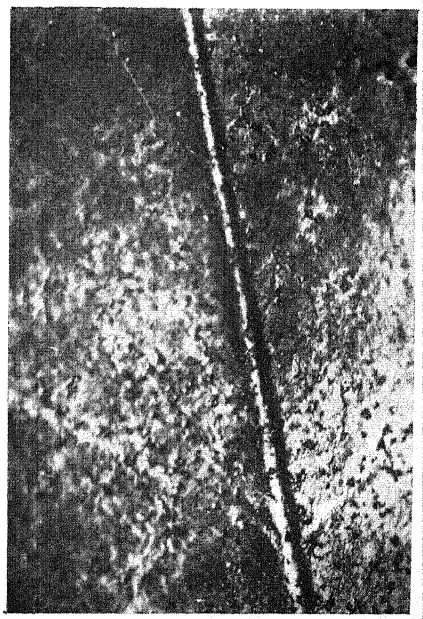
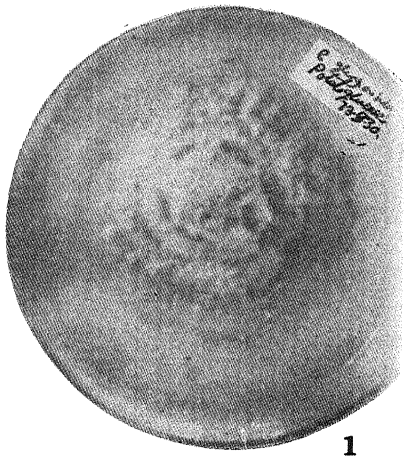


FIG. 4. Acervuli of *C. gloeosporioides* on *citrus* leaf produced by artificial inoculation. (×5.)



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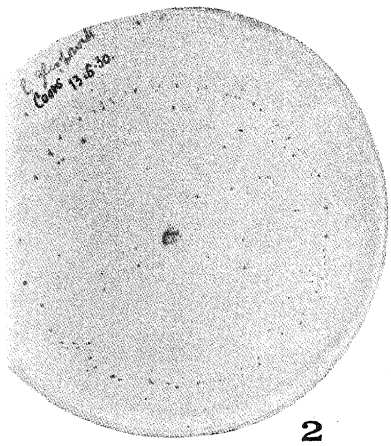

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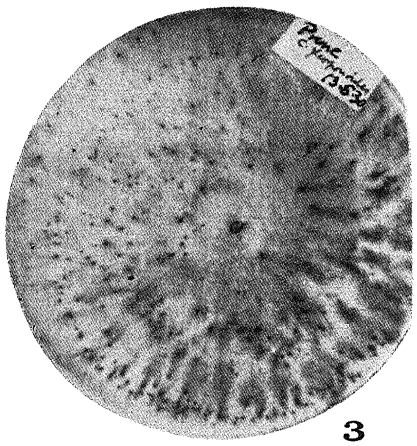

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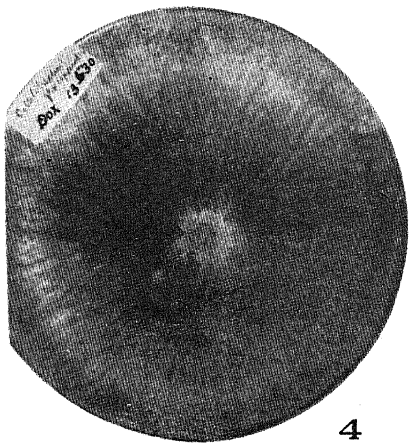

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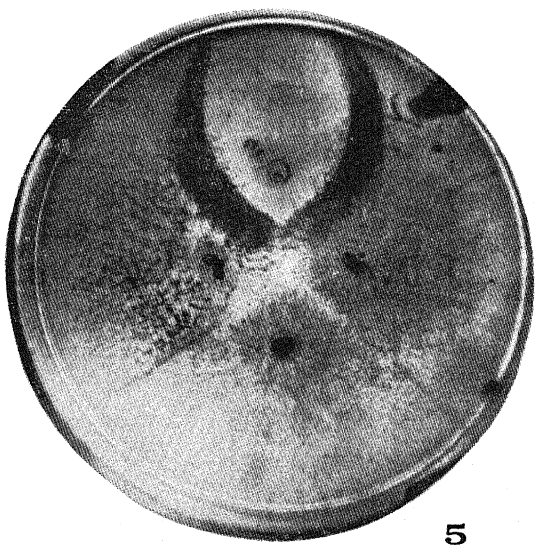

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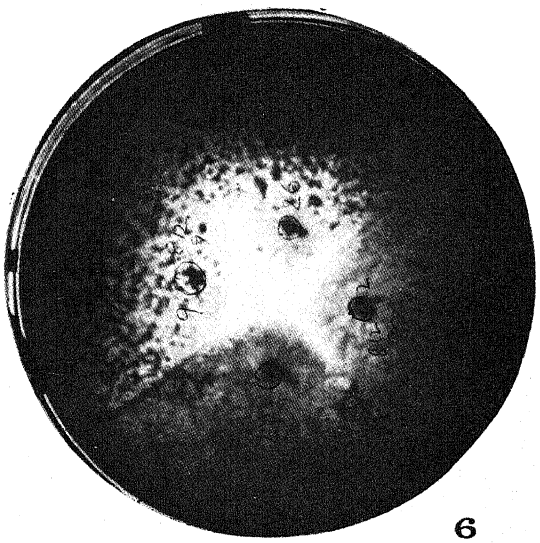
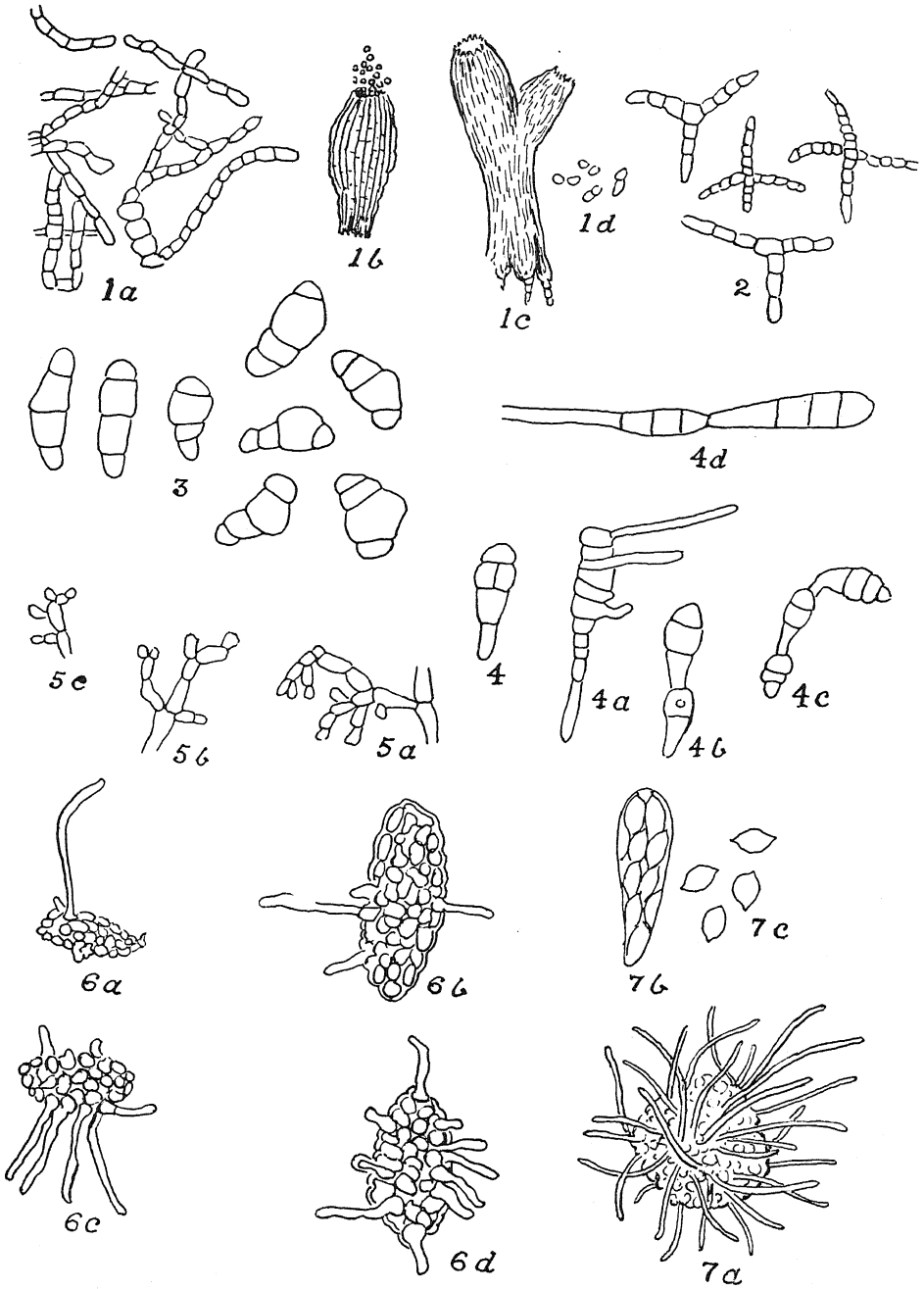


6

FIGS. 1, 2, 3, 4. Growth character of a strain of *C. gloeosporiodies* on four different media. (×2/3.)

FIGS. 5, 6. Four different strains of *C. gloeosporioides* inoculated at four different points of the Petri dish.

Note the space of aversion, white line of aversion and complete fusion in different cases. (×7/9).



SOOTY MOULD OF *CITRUS*.

FIGS. 1 and 2, *Capnodium citri*.

1. (*a*) Hyphae. Note some are segmented. (×480.) (*b*), (*c*), (*d*). Pyncidia and spores (×350).
2. Spores of *Triposporium* form (×350).

FIG. 3. Spores of *Acrothecium lunatum* (×610).

FIG. 4. Spores of *Alternaria citri* (×610).

In (*a*) hyphae being formed from each segment of the spores. In (*b*), (*c*) and (*d*) spores are being formed in chains.

FIG. 5. *Cladosporium herbarum* var. *citricola* (×750). Branching conidiophores bearing conidia.

FIG. 6. *Pleospora herbarum*, a-d (×450) germinating ascospores.

FIG. 7. *Chaetomium* sp. (*a*) Perithecia (×125), (*b*) ascus with ascospores (×650), (*c*) ascospores (×650).

FIG. 1. A twig of *malta* showing injury caused by hail-stone which later became infected by *C. gloeosporioides*.

FIG. 2. Anthracnose spots on leaves of *khatta*.

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A NEW VARIETY OF *OIDIOPSIS TAURICA*

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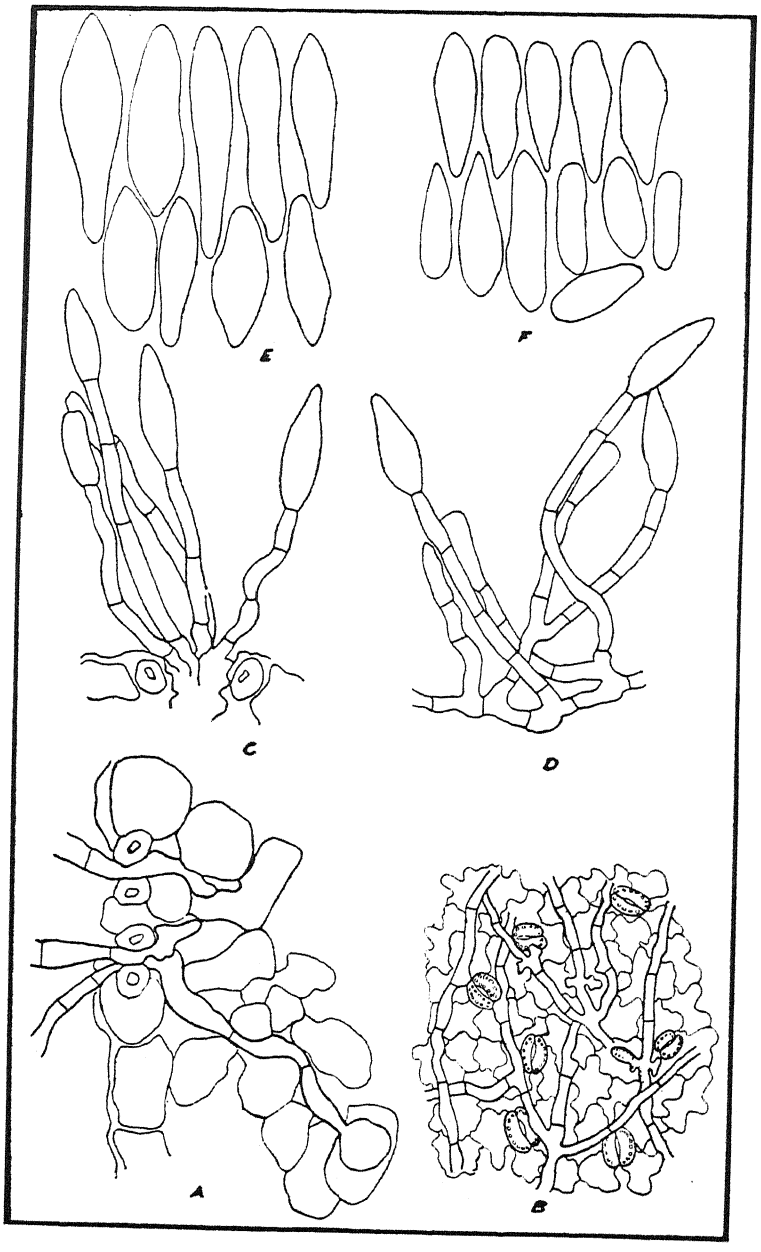
(With Plate XX and one text-figure)

In 1933 powdery mildew was observed on a single vine of *Dolichos lablab* L. in the grounds of Fergusson College in Poona. In general characteristics the fungus showed a close resemblance to *Oidiopsis taurica* (Lév.) Salm., which is a common parasite on a variety of plants in the Bombay Presidency. The conidia of the *Dolichos* fungus, however, were of unusually large size, and this character seemed to separate it from *Oidiopsis taurica*. The results of a study of this fungus are briefly described in the following pages.

PATHOLOGICAL EFFECTS OF THE FUNGUS ON THE HOST

In general, the pathological effects produced by the fungus on *Dolichos lablab* are very similar to those produced by *Oidiopsis taurica* on cluster bean, *Cyamopsis psoraloides* DC. The fungus forms on the upper surface of the leaves dull red spots, which are irregular in outline and are limited by leaf veins. On the under-surface of the leaves, white powdery patches of mildew are observed corresponding to the extent of the spots. The leaf dries up in the infected region, but in some cases the local discolouration associated with separate infected areas may become obliterated in the general invasion and the consequent drying up of the whole leaf. The affected plants are defoliated.



A. Transverse section of an affected leaf of *Dolichos lablab* showing endophytic mycelium ramifying in the intercellular spaces and in the sub-stomatal vesicles, and sending globular haustoria into the mesophyll cells. *B*. Portion of an affected leaf showing external mycelium, which possesses appressoria-like structures. *C*. Conidiophores emerging in fasicles through a stoma and bearing on their ends a single conidium. *D*. Branched conidiophores. External mycelium arising as lateral branches from the main axis of conidiophores. *E*. Conidia of *Oidiopsis taurica* var. *macrospora* showing range of shapes and sizes. *F*. Conidia of *Oidiopsis taurica* showing range of shapes and sizes.

MORPHOLOGY OF THE FUNGUS

The fungus at first is wholly endophytic. The mycelium ramifies in the inter-cellular spaces of the spongy and palisade parenchyma, and sends into the mesophyll cells small globular haustoria. The hyphae are septate and tortuous, and are abundant just below the epidermis and in the sub-stomatal vesicles. The epidermal cells are not invaded by the fungus (Plate XX, A).

From the internal mycelium, conidiophores arise and emerge singly or in fasicles through the stomatal apertures. The conidiophores are septate, sometimes simple, but more often branched, and are variable in length measuring up to 280 μ. They bear on their ends a single conidium. From the main axis of the conidiophores, lateral branches sometimes arise and spread indefinitely, forming the external mycelium (Plate XX, C and D). The hyphae of the external mycelium also emerge through the stomata, and in the later stages of the conidial phase, the mycelium is persistent, effused and often densely compacted, giving the appearance of white mealy growth. Sometimes short conidiophores may arise from the external mycelium (Plate XX, D). No haustoria are sent into the epidermal cells from the external mycelium, which nevertheless possesses structures similar to appressoria on the superficial mycelium of ectophytic powdery mildews (Plate XX, B). The external mycelium also forms feeding branches, which enter through the stomata to obtain nourishment from the inside of the leaf.

The conidia are extremely variable in shape and size. They are sub-cylindric, often more or less sub-acuminate towards the apex, or irregularly shaped with the middle part of the spore swollen or constricted, single-celled, and measure 47 to 111 μ × 10 to 34 μ (Plate XX, E). The conidia germinate by the formation of hyphae, which enter the host through the stomatal apertures and send haustoria into the mesophyll cells of the leaf. The germ-tube forms appressoria like the external mycelium.

The perithecial stage of the fungus was not found.

CROSS-INOCULATION EXPERIMENTS

Curiously enough, the fungus on *Dolichos lablab* has been observed in nature on one or two vines only, and extensive search has failed to reveal it elsewhere. Evidence has been obtained that *Oidiopsis taurica* is highly specialised in its parasitism, and it is therefore difficult to determine whether the larger size of the conidia of the fungus on *Dolichos lablab* is due to the host influence. The sudden appearance of *Oidiopsis* on *Dolichos lablab* cannot be explained at present, and the work on host specialisation in *Oidiopsis taurica*, the results of which will be reported elsewhere, has also given no indications of the presence of any bridging

hosts. The fungus on *Dolichos lablab*, however, is capable of infecting this host under all conditions. The results of some cross-inoculation experiments are summarised in Table I.

TABLE I

Summary of results obtained by inoculating different hosts with conidia of Oidiopsis taurica *and of* O. taurica *var.* macrospora

Hosts inoculated	Conidia taken from			
	Dolichos lablab	*Cyamopsis psoraloides*	*Oxalis corniculata*	*Euphorbia geniculata*
Dolichos lablab . . .	*5/4	14/0	5/0	8/0
Cyamopsis psoraloides . .	7/0	13/11	5/0	9/0
Oxalis corniculata . . .	2/0	5/0	5/3	8/0
Euphorbia geniculata . .	2/0	9/0	3/0	9/7

* The numerator represents the number of plants inoculated, and the denominator the number of plants infected.

TAXONOMY

Salmon [1906] emended the genus *Oidiopsis* of Scalia to include those members of the Erysiphaceae which are chracterised by their completely endophytic habit during the early part of the conidial phase and produce branched conidiophores emerging singly or in bundles through the stomata. The fungus then known as *Erysiphe taurica* Lév. was transferred to this genus as *Oidiopsis taurica* (Lév.) Salmon. This species shows a wide variation in the size and shape of conidia and has been recorded on a large variety of host plants. The shape of the conidium, however, is often sufficiently constant and distinctive and does not vary as widely as the size.

As has been pointed out above, there is no evidence to show that the size and shape of the conidia of *Oidiopsis taurica* is affected to any appreciable extent by the host, although, as suggested by Salmon [1906], it seems probable that the variation shown by the conidia may be due to their position on the conidiophore.

The wide variation exhibited by the conidium is nevertheless sufficiently constant to make the fungus *O. taurica* easily recognisable. Salmon [1906] has, however, recognised a variety, *O. taurica* var. *lanuginosa* (Feckl.), on the basis of variation in the shape of the conidium.

Although the conidia of the fungus on *Dolichos lablab* fall within the range of normal form in shape usually encountered in *Oidiopsis taurica* (Plate XX, E and F), their size presents a feature most sharply distinguishing the *Dolichos* fungus. Fig. 1 and Table II will show that the conidia of *Oidiopsis* on *Dolichos lablab* are substantially longer than those of *O. taurica*, although there is not much difference in width. The conidia of the *Dolichos* fungus measure from 47 to 111 μ in length, whilst the range of length of the conidia of *O. taurica* as given by Salmon is from 32 to 82 μ.

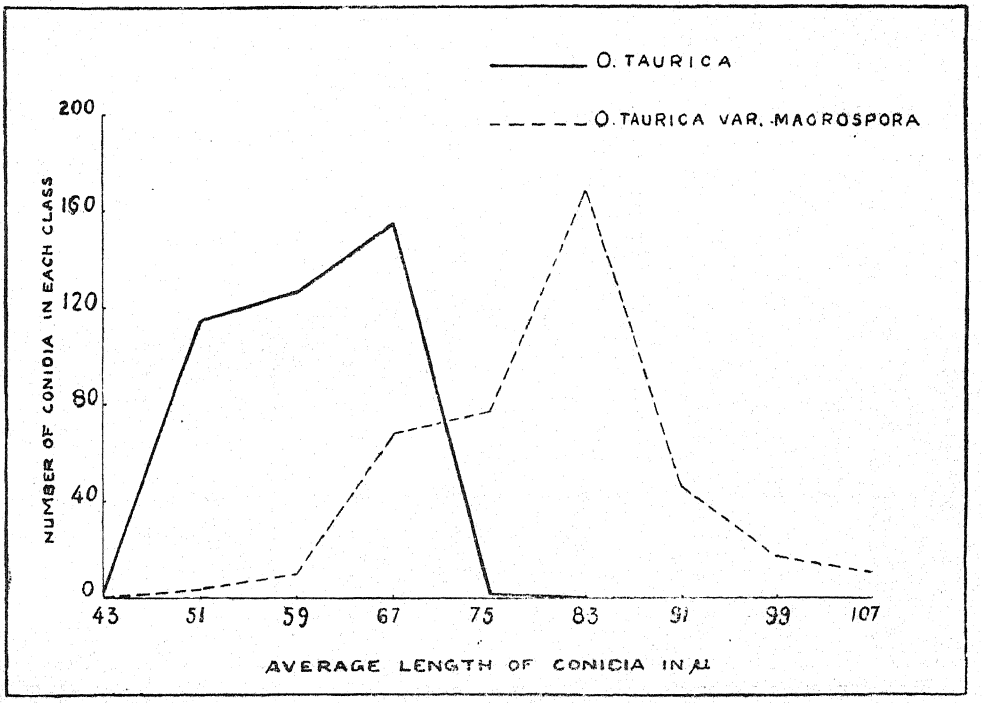


Fig. 1. Measurements of conidia of *Oidiopsis* on *Dolichos lablab* and those of *O. taurica*.

TABLE II

Comparative measurements of conidia of Oidiopsis taurica *on* Cyamopsis psoraloides *and of* O. taurica *var.* macrospora *on* Dolichos lablab

Classes in μ	Length		Classes in μ	Width	
	Number of conidia in 400			Number of conidia in 400	
	Dolichos lablab	*Cyamopsis psoraloides*		*Dolichos lablab*	*Cyamopsis psoraloides*
39 to 46·9 .	0	2	4 to 9·9	6	0
47 to 54·9 .	4	115	10 to 15·9	54	123
55 to 62·9 .	10	127	16 to 21·9	271	277
63 to 70·9 .	67	153	22 to 27·9	67	0
71 to 78·9 .	77	2	28 to 33·9	2	0
79 to 86·9 .	168	1	..	..	..
87 to 94·9 .	46	0	..	..	..
95 to 102·9 .	17	0	..	..	..
103 to 111 .	11	0	..	..	..

DIAGNOSIS

The *Dolichos* fungus differs from *Oidiopsis taurica* in the length of the conidium, a character which is constant and sufficiently distinctive to justify its separation as a variety. The name proposed for the new variety is intended to be descriptive of the large size of its conidia, thus emphasizing the important disgnostic character of the fungus.

Oidiopsis taurica var. *macrospora* var. *nov.*

External mycelium hypophyllous, usually persistent, effused, densely compacted, white. Internal mycelium ramifying in the inter-cellular spaces between the mesophyll cells and sending into these cells small globular haustoria.

Conidiophores septate, sometimes simple, but more often branched, emerging singly or in bundles through the stomata, measuring up to 280 μ, bearing single conidium on the end, also arising from the external mycelium, which possesses short appressoria-like structures.

Conidia extremely variable in shape and size, sub-cylindric, often more or less sub-acuminate towards the apex, or irregularly shaped with the middle part of the spore swollen or constricted, single-celled, measuring 47 to 111 μ × 10 to 34 μ, germinating by hyphae which enter the host through stomata and send haustoria into mesophyll cells of the leaf.

Attacking leaves of *Dolichos lablab* L., on the upper surface of which producing dull red spots and mealy white growth on the lower surface in areas corresponding to the spots, the leaf later drying up in the infected region. On one or two plants in the grounds of Fergusson College in Poona.

Type material deposited in the herbaria of the College of Agriculture, Poona, and of the Imperial Mycological Institute, London.

Summary

The fungus causing powdery mildew on *Dolichos lablab* has been described as a new variety of *Oidiopsis taurica*, from which it differs by the large size of its conidia. The name proposed for the *Dolichos* fungus is *Oidiopsis taurica* var. *macrospora*. The fungus produces dull red spots on the upper surface of the leaves of *Dolichos lablab*, the corresponding areas on the lower surface supporting white mealy fungus growth. This fungus has been observed on one or two plants only of *Dolichos lablab* in Poona.

The writers gratefully acknowledge the help received from Mr. S. F. Ashby, Mycologist, Imperial Mycological Institute, London.

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MICROSPOROGENESIS IN *CROTALARIA JUNCEA* LINN.

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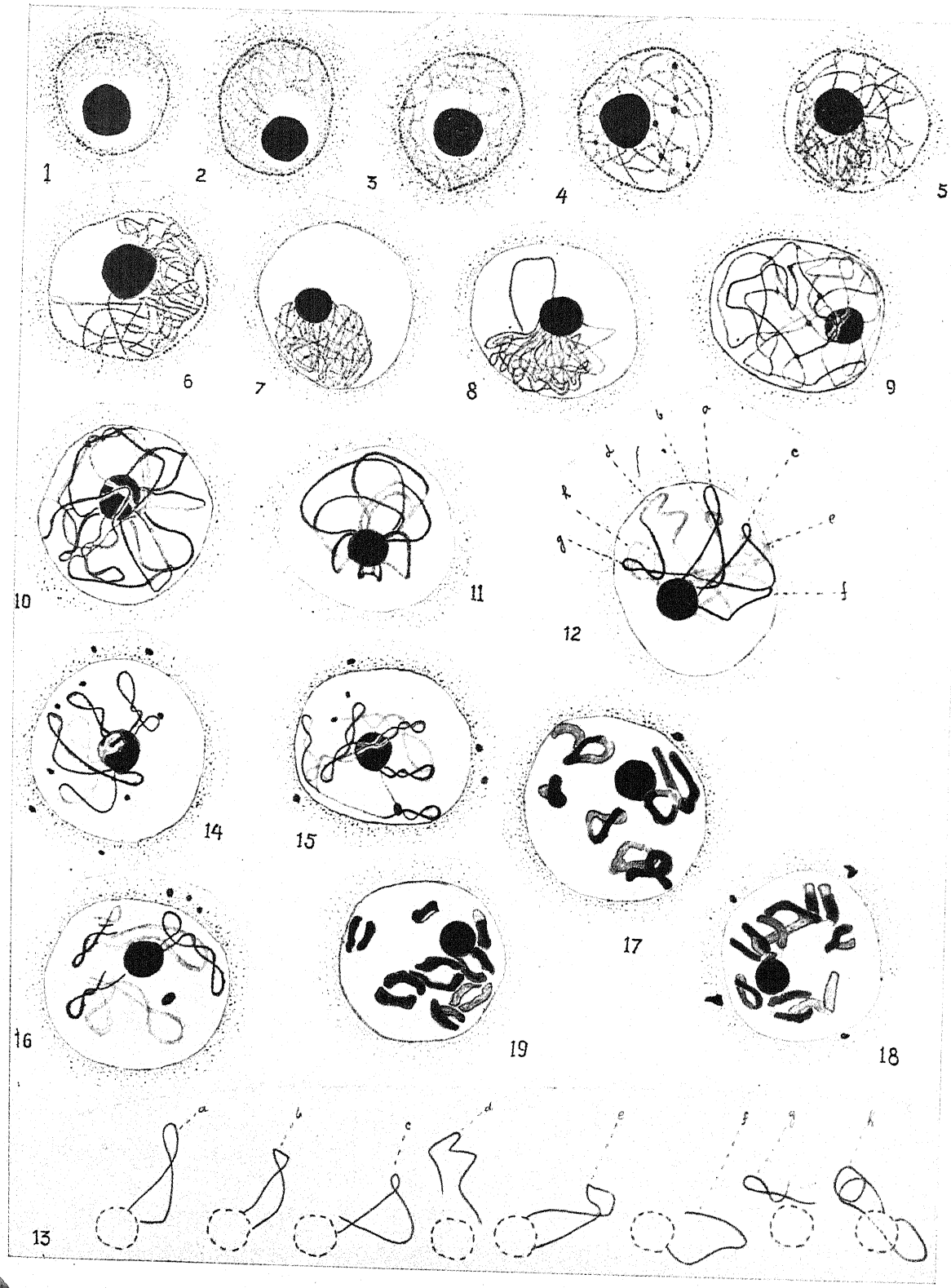
(With Plates XXI and XXII)

The family Leguminosae contains nearly twelve thousand species, distributed all over the earth. Considering the large number of its species and their economic importance very little cytological and genetical work has as yet been carried out. Herbaceous plants of this family are commonly used as green manures and *Crotalaria juncea* Linn. is one of them. *Crotalaria juncea* is also one of the important fibre-yielding plants.

Microsporogenesis

The development of the pollen grains with reference to synizesis and chromosome conjugation in the family Leguminosae has received very little attention from cytologists. Latter [1926] worked on *Lathyrus odoratus* and suggested a telosynaptic method of chromosome conjugation. Her observations have, however, been criticised later by Maeda [1930]. Weintein [1926] worked out the cytology of *Phaseolus vulgaris*. He found the close approximation of two threads in late prophase. The segmentation of these threads gave rise to the bivalent chromosomes. Sethi [1930] working on *Cassia didymobotrya* found the spireme to be connected with the nucleolus and suggested a transference of material from the latter to the former. Reeves [1930] has worked out recently the microsporogenesis of *Medicago sativa*.

A comprehensive account of the chromosome number belonging to plants of this family has been recently given by Tischler [1931]. The chromosome number of *C. juncea* has been determined by many workers. Ramanujam, Parthaswarthy and Ramiah [1933] found eight to be the haploid number in four species of *Crotalaria*. Datta [1933], however, reports ten as the haploid number in *Crotalaria juncea*.



(For explanation see page 125)

Material and methods

Plants for this investigation were grown in the College experimental garden, and flower buds of various ages were fixed in the field on bright sunny days. Fixation between 12 noon and 4 P.M. gave best results. To facilitate the penetration of the fixing fluid an exhaust pump was used at the time of fixation, or the material was first dipped in acetic alcohol (1 : 2) for a short time before immersion in the fixatives. A number of fixing fluids were tried, but satisfactory results were obtained from flower buds fixed in Allen's modified Bouin's fluid. The material was dehydrated, cleared and embedded in paraffin in the usual way.

Microtome sections were cut of various thickness ranging from 8 to 16 μ according to the object of study. Sections were stained chiefly in Heidenhain's Iron-alum haematoxylin with or without a counter stain. Newton's Iodine Gentian violet stain was also used.

The resting nucleus

The last premeiotic division takes place rather rapidly, and in the same loculus preparatory stages for meiotic division and the resting pollen-mother cells can be observed. In the resting stage the pollen-mother cells are polygonal in outline and are closely compressed inside the anther loculus, there being no intercellular spaces. Each cell is bounded by a thin wall and is filled with finely granulated cytoplasm without vacuoles or dark staining bodies. The nucleus occupies more or less the central position.

The resting nucleus of the pollen-mother cell is nearly spherical in outline with a hardly distinguishable nuclear membrane. A large globular, deep staining nucleolus with a perinucleolar hyaline zone, occupies the central position. The hyaline zone is hardly traversed by peripheral granular strands (Plate XXI, fig. 1). The invariable occurrence of the perinucleolar hyaline zone, even in well-fixed material gives little support to McClung's [1929] contention that, it is due to the shrinkage of the nuclear contents away from the nucleolus because of the inadequate fixation of the karyolymph.

Heterotypic prophase

The activity of the nucleus starts with the appearance of a faintly staining granular reticulum at the periphery (Plate XXI, fig. 2). The reticulum gradually becomes coarser and the nucleolus also increases a little in size (Plate XXI, fig. 3).

Leptonema stage

The reticular condition is directly followed by the leptonema stage in which the network resolves into a fine continuous thread of granular appearance and is spread uniformly throughout the nuclear cavity (Plate XXI, fig. 4). The perinucleolar hyaline zone so far distinct is now lost sight of. No doubleness of the

thread nor any free end is observed at this stage. Some deep staining dots are always seen in association with the thread. These are slightly bigger than the net-knots which are formed by the crossing of several threads at a point, and cannot be considered as such. Their sudden appearance at this stage and disappearance abruptly in synizesis leads one to believe that their presence is due to some metabolic changes taking place in the protoplast at this stage.

Synizesis

The first indication of synizesis is seen by the withdrawal of the threads from the periphery towards the centre. The network gradually contracts uniformly from one side of the cell to the other, while the nucleolus retains its original position (Plate XXI, fig. 5). The entire network passes to one side of the nucleolus and remains as a contracted mass along the nuclear membrane without enclosing the nucleolus in its meshes (Plate XXI, fig. 6). It is not clear how the nucleolus escapes out of the reticulum and comes to lie at one side of the synizetic knot. It is too big to pass through its meshes and too viscous to be filtered through the net. Possibly the thread separates into two parts to allow the nucleolus to escape, but in the absence of corroborative evidence it is difficult to say anything definitely. The relative position of the nucleolus and the synizetic knot remains the same. The separation of the thread from the nucleolus is never complete and the knot is not a tight one, but is rather loose and the thread can be traced for a considerable distance before it becomes obscure (Plate XXI, fig. 7).

The later prophasic changes clearly show that chromosome pairing in this case is typically telosynaptic and so the actual significance of synizesis as defined by parasynaptists "the closer association of the homologues by their rearrangement" does not hold good here. But the synizetic knot in this case cannot be considered to be an artifact caused by the coagulation of the karyolymph as suggested by McClung [1929] or by its plasmolysis as Chodat [1929] suggests, since all the materials properly fixed with different fixatives present the same appearance of the synizetic knot. Sargant [1897] noticed the invariable presence of this stage in well-fixed material and even in living material. Though synizesis seems to be an essential stage through which the spireme passes in meiosis, its actual significance in some cases is open to question, and its absence again in some species of Oenothera is peculiar and challenges the essentiality of such a stage.

Open spireme

The contracted spireme opens out gradually by throwing irregular loops towards the distal end (Plate XXI, fig. 8). The thread is rather uniform in appearance, but very slender and does not stain deeply. Following this stage the pollen-mother cells gradually lose their polygonal shape and round off. When the synizetic knot is fully unravelled it fills up the entire nuclear cavity. A

typical hollow spireme results and the thread is disposed inside the nucleus in large irregular coils (Plate XXI, fig. 9). No free end of the thread (except the cut ends), nor their dual nature is clear at this stage. Whenever free ends are seen they are invariably at focal extremes suggesting them to be cut ends of a continuous spireme, the remaining segments in the consecutive sections justifying this contention.

Pachynema

The thread thickens up by contraction and the loops become more conspicuous (Plate XXI, fig. 10). The loops are so wide that it is difficult to count their number accurately, but careful observation shows that they do not exceed the haploid number of chromosomes of the plant. The continuity of the thread is very clear, no free ends being visible, and it is uniformly thickened all through. The nuclear cavity is very much expanded and the mother cells round off completely and become mostly independent of one another.

Second contraction or brochonema

The spireme gradually condenses and the number of loops become distinct (Plate XXI, figs. 11 and 12). As far as can be made out the loops are continuous with one another. The number of persistent loops is the same as the haploid number of chromosomes, suggesting as if each loop is composed of a pair of chromosomes joined end to end. The folds of the thread forming the loops lie mostly about the nucleolus and when not so, the central knot is not too far away from it. Further condensation of the thread produces a characteristic orientation of the loops. It appears as if the loops are radiating from the centre where the nucleolus is situated.

The loops twist in various ways turning through different angles round their own axis. The nodes or chiasma so formed by the twisting of the loops suggest at once as if they bring about an interchange of genes between two components of a pair of homologues, which is otherwise insufficient by their union at one place at the end. This interpretation gets more support due to less number of nodal regions in the smaller pair, where in comparison with their smaller size only one point of union is sufficient for the interchange. The number of chiasma so far obtained is a constant character for a particular pair.

Early diakinesis

Further contraction of the spireme leads to the segmentation of the loops from the knot but the actual mechanism of this process is not clearly understood (Plate XXI, fig. 16). Some of the loops which lie away from the knot are seen still to be connected with it by a fine strand of dark staining material (Plate XXI, fig. 15), and if this strand be taken as a part of the true chromosome-forming

substance which is drawn into its total composition afterwards, then the mechanism of breaking can be explained as due to the contraction of the chromatin of each loop separately from the knot, and the ground matrix of the chromosomes is left over forming the connecting link. This link gives way later being less tenacious and thus the separation of the loops is brought about. This view gets support from the evidence obtained in the late-diakinetic stages where chromatin of each chromosome contracts leaving a hyaline connection at the tip of the previous loop, and therefore it can be suggested that the segmentation of the spireme is due to the condensation of the chromatin of the homologous pair whereas the separation of the homologues is by the contraction of the individual chromosomes. If on the other hand the thin strand be considered as not taking any essential part in the formation of the chromosomes but later dissolves freely in the nuclear cavity, then the mechanism of segmentation of the loops can be considered from a different point of view, *viz.*, the dissolution of the chromatin of each loop near the knot. This transference of chromatin material from the chromosomes to the karyolymph is evidenced by the fact that the nuclear cavity now contains some deep staining material. But the simultaneous occurrence of similar deep staining bodies in the cytoplasm goes against this view (Plate XXI, figs. 14-18).

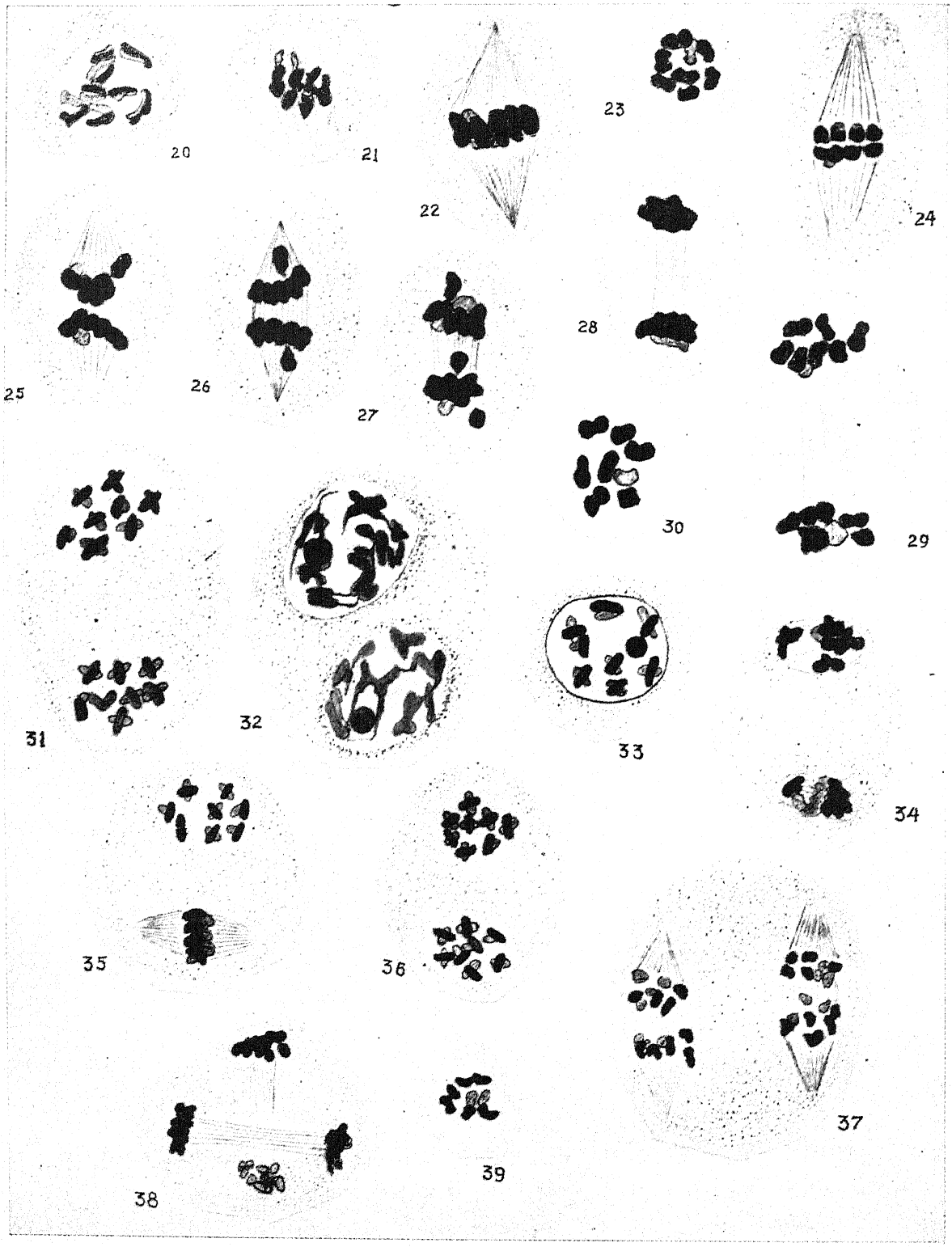
Diakinesis

When the segmentation of the loops is complete, the separate pairs lie scattered in the nuclear cavity. The two arms of each loop separate out and they appear to be very much thickened. The two free ends of each pair, so far quite apart, now come to lie closer together, causing them to appear as rings or figure 8. Besides the forms 0 and 8, they often assume the forms of V and Y (Plate XXI, figs. 17-19). The figure 8 form may be due to the twisting of the rings or due to the unfolding of the primary twists of the loops. The different pairs can be classified according to their lengths even at this stage of condensation, and this gives the graphic formula $2S + 5M + 1L$

Further condensation of the individual chromosomes of a homologous pair throws much light on their mode of conjugation. The chromatin of each pair contracts with a definite sequence from the point of union of the two homologues leaving a hyaline zone at the tip of the loop suggesting thereby that the paternal and the maternal chromosomes are joined end to end and their point of union is at the tip of the brochonema loops.

Plate XXI, fig. 18 shows some of the chromosomes with hyaline zone at the point where they are attached to their homologues. This shows that the chromosomes are composed of two substances : the basophilic chromatin and another hyaline ground substance which most probably forms the dispersion medium.

The two arms of the bivalents thus break apart at their point of union and the two components either lie in a ring or side by side in close approximation (Plate XXI, fig. 19).



(For explanation see page 125)

The deeply stained nuclear and cytoplasmic bodies, which were at first noted in the early-diakinetic stage and were present so far, now disappear from all parts of the cell.

Spindle formation

The nuclear membrane disappears abruptly before any spindle fibres are noted either as polar caps or inside the nuclear cavity. It is clear that the nuclear membrane becomes gradually thinner in the late-diakinetic stage. With the disappearance of nuclear membrane the scattered chromosomes clump together in the centre surrounded by a hyaline zone. This hyaline area is somewhat coarser and striated, suggesting intra-nuclear origin of spindle fibres (Plate XXII, fig. 20).

The components of a homologous pair establish more intimate contact and no clear space is evidenced in between them, but they still retain their rod-shaped structure. No multipolar spindle is observed. The spindle when first noted shows the fibres arranged in straight rows (Plate XXII, fig. 21), but when its bipolar nature is completely established, some of the fibres appear to be considerably thicker, specially towards the poles. They are fewer in number and their actual attachment to the chromosomes is not clear. The spindle formation is so rapid that stages from diakinesis to heterotypic metaphase are observed in the same loculus.

Heterotypic metaphase

The gemini which clumped in the centre of the nucleus separate out and their bivalent nature is apparent even in their maximum stage of condensation. Polar view of an equatorial plate shows eight such bivalents indicating thereby that the haploid number is eight (Plate XXII, fig. 23). The spindle now shows distinct polarity and the converging fibres meet at two sharp points. The bivalents are arranged lengthwise along the equatorial region of the spindle with each component towards each pole, the constriction between them being in the centre (Plate XXII, fig. 22). The nuclear zone formerly unoccupied by the cytoplasm and so long distinct is now encroached upon by the cytoplasm. But the cytoplasm surrounding the spindle remains less dense than the peripheral zone of the cell.

Heterotypic anaphase and telophase

The constriction of the bivalents in the equatorial zone of the spindle now becomes more prominent and well-marked split appears (Plate XXII, fig. 24). The chromosomes now disjoin and the univalents, spherical in shape, move towards the poles. Their movement to the poles is not very regular, one chromosome belonging to each haploid set moves earlier (Plate XXII, figs. 25 and 26)

and reaches the pole before the rest (Plate XXII, fig. 27). In some cases one or two chromosomes are seen to lag behind the rest. When the chromosomes reach the poles, they clump together and their individuality cannot be made out (Plate XXII, fig. 28).

Before the disappearance of the spindle fibres, the chromosomes get separated from one another and look distinct in a telophasic plate (Plate XXII, figs. 29 and 30). The homotypic split of the univalent chromosomes which could not be made out even in the late anaphase now appears all on a sudden and in some the split is quite apparent.

In late telophase the spindle fibres completely disappear but a fine cytoplasmic zone is left round about the polar region. The split becomes complete and the dual nature of each univalent chromosome is evident. Most of the univalents have the two split-halves lying side by side or in the form of a cross (Plate XXII, fig. 31). In the latter case this is probably due to the separation of the two halves from one another at two ends, being connected only at the central region, so that one half swings across the other approximately at right angles to it. Previous to this, when the split was not complete but the dual nature was prominent, the constriction appeared to be transverse, due probably to the extreme condensation of the chromosomes. This apparent transverse constriction cannot be otherwise interpreted, because of the spherical nature of the univalents at this stage. It is a well-known fact that the plane of division of a sphere is always transverse. But from the relative sizes and parallel association of the components of the univalents, it is clear that the split is a longitudinal one and not transverse.

Interkinesis

A clear zone appears surrounding the chromosome region; most probably this is due to the deposition of the nuclear sap; and the nuclear membrane appears simultaneously with the appearance of the nucleolus. The size of the nucleolus is very small at this stage. The chromosomes elongate very much, sending out chromatin strands which intertwine and anastomose freely between them (Plate XXII, fig. 32). The chromosomes do not resolve into complete threads and their number can be easily counted, though they do not retain their original form and their double nature is indistinct.

Homotypic division

Before the formation of the homotypic spindles the nuclear membrane becomes thinner. Very faint fibrillar structures appear in between the chromosomes, suggesting intra-nuclear origin of the homotypic spindles (Plate XXII, fig. 34). The nucleolus is not clearly seen at this stage, due to clumping of the chromosomes. Later the membrane disappears completely and the chromosomes

become distinct from one another. No trace of the nucleolus is observed. The homotypic spindles are always bipolar but their space relationship is very variable, they may lie parallel or at right angles to each other (Plate XXII, figs. 35 and 36).

The univalent chromosomes in a homotypic equatorial plate appear mostly as crosses and a few as V or Y (Plate XXII, fig. 36). The appearance presented at this stage is very similar to that observed in the late telophase before the organisation of the inter-kinetic nucleus (Plate XXII, fig. 31).

The chromosome halves, already split, move apart and reach the poles very quickly. The chromosomes now appear spherical without any split or constriction and all of them do not move to the pole with the same velocity, some moving earlier and some lagging behind (Plate XXII, fig. 37).

Spindle fibres do not disappear until the individual chromosomes in the telophase get separated from their aggregated condition formed in the early telophase (Plate XXII, fig. 38). A polar plate of this stage shows the appearance of constriction in the chromosomes (Plate XXII, fig. 39). This split is a preparation for the first division in the microspore.

A clear zone appears in the polar region due to the deposition of the nuclear sap, and the nuclear membrane appears simultaneously with the appearance of the nucleolus. No spindle fibres nor any trace of them are observed at this stage. The chromosomes resolve into chromatin threads but not completely until cytokinesis begins. Thus the tetrahedral arrangement of the granddaughter nuclei is established and an examination of a large number of such stages shows that this is not always due to the decussate arrangement of the spindle fibres.

The nucleus gradually increases in size and the chromosomes anastomose with one another sending chromatin strands in all directions. They lose their deep staining property and the appearance of such anastomosis is an indication of the formation of a resting reticulum.

Cytokinesis takes place by the process of furrowing. The microspores are liberated by the dissolution of the thickened mucilaginous layer. The mature pollen grains are somewhat elongated and uni-nucleate. The pollen grains of the linear and globose anthers are similar in shape and size.

Summary

1. The resting nucleus of the pollen-mother cell shows a granular zone along the peripheral region and a hyaline perinucleolar area.

2. The spireme remains granular in the leptonema and synizetic stages.

3. The spireme is continuous all through. No longitudinal split or parallelism of threads has been observed.

4. Brochonema loops are equal to the haploid number of chromosomes.

5. Diakinesis is normal and the bivalents exhibit the following forms :— V, O, X and Y. In the late diakinesis the components of each homologous pair contract leaving a hyaline connection at their point of union.

6. Some deeply staining bodies occur in early-diakinetic stage simultaneously both inside and outside the nucleous. These disappear in the late diakinesis.

7. Absence of parallelism of the leptonema and pachynema threads, the typical brochonema stage with the loops equal to the haploid number of chromosomes and the separation of each loop into two univalents suggest that the method of chromosome pairing is telosynaptic, where the paternal and the maternal chromosomes are joined end to end in the spireme.

8. The heterotypic metaphase is normal. In anaphase two chromosomes are seen to move faster than the rest, one to each pole.

9. Polar view of an equatorial plate at heterotypic metaphase shows eight bivalent chromosomes.

10. Homotypic split is first noticed in heterotypic telophase and the split is so complete that the chromosomes appear in the forms of X, V or Y.

11. There is a very short interphase between heterotypic and homotypic divisions.

12. The origin of both heterotypic and homotypic spindles appear to be intra-nuclear.

13. Homotypic division is normal. The space relationship of the spindles is variable.

14. Cytokinesis takes place by furrowing.

15. Mature pollen grains are uni-nucleate and lie scattered in the loculus.

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Explanation of Plates XXI and XXII

The figures were drawn with the aid of a Camera lucida at table level. The magnification of each of the figures is 2,400.

PLATE XXI

Fig. 1. A resting pollen-mother cell with a granular peripheral zone and a hyaline perinucleolar zone.
Fig. 2. Very early prophase ; early indication of thread formation is evident.
Fig. 3. Advanced reticulum.
Fig. 4. Leptonema. Thread single and univalent.
Fig. 5. Early synizesis. The reticulum contracts from the nuclear membrane.
Fig. 6. The massing of the reticulum to one side of the nuclear cavity.
Fig. 7. Typical synizesis.
Fig. 8. Loosening of the spireme after synizesis. No indication of the granular appearance of the thread remains.
Fig. 9. Hollow spireme.
Fig. 10. Pachynema.
Figs. 11 & 12. Second contraction. Eight radiating loops are seen.
Fig. 13. The eight persistent loops of the previous figure are drawn separately, showing the position of each with regard to the central nucleolus.
Fig. 14. Later stage of second contraction.
Fig. 15. Segmentation of the loops to form the bivalent chromosomes.
Fig. 16. Early diakinesis.
Fig. 17. Diakinesis.
Fig. 18. Contraction of the individual chromosomes of the gemini resulting in the partial separation of the univalents.
Fig. 19. Side-by-side association of the homologues after their separation (composite drawing from two sections).

PLATE XXII

Fig. 20. Alignment of the chromosomes in the equatorial plate prior to the formation of the spindle. The nuclear membrane and the nucleolus have completely disappeared.
Fig. 21. First appearance of the bipolar spindle.
Fig. 22. Heterotypic metaphase.
Fig. 23. Polar view of an equatorial plate showing " eight " bivalent chromosomes.
Fig. 24. Early anaphase. Note separation of the univalents.
Figs. 25 & 26. Anaphase. Note early migration of a single chromosome to each pole.
Fig. 27. Late anaphase. Two chromosomes stand out separate at the poles. They have reached earlier.

Fig. 28. Telophase.
Fig. 29. Separation of the individual chromosomes at telophase.
Fig. 30. Polar view of a telophasic plate. Appearance of the homotypic split in the chromosomes just apparent in this stage.
Fig. 31. Late telophase. Components of univalents appear mostly as crosses.
Fig. 32. Interkinesis. Note the drawn out condition of the chromosomes.
Fig. 33. A late stage showing eight split chromosomes.
Fig. 34. Intra-nuclear origin of the homotypic spindle.
Fig. 35. Homotypic metaphase.
Fig. 36. Polar view of homotypic metaphase. Note the split condition of the chromosomes.
Fig. 37. Homotypic anaphase.
Fig. 38. Homotypic telophase.
Fig. 39. Chromosomes in telophase showing their constricted nature.

RESEARCH NOTE

EFFECTS OF BROWN RUST ATTACK ON WHEAT

BY

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(Received for publication on 31st October 1935)

Johnston and Miller (*J. Agric. Res.* 1934 : **49** : 955-81) studied the effect of brown rust infection on the growth, yield and water economy of Warden and Pusa 4 wheats. Physiologic form 9 of brown rust (or leaf rust or orange rust as it is alternatively called) was used in these investigations. Pusa 4 is susceptible to this physiologic form while Warden is highly resistant to it.

Seeds were sown in sealed one-gallon stone jars. For each variety there were five groups of six jars containing six plants each. One group of each variety was maintained to maturity as a rust-free control while the remaining four groups of each variety received their initial inoculation with brown rust at the seedling, jointing, booting* and flowering stages, respectively. After this each inoculated group was re-inoculated sufficiently often to ensure abundant infection.

The data show that brown rust infection greatly reduced the yield of grain in the susceptible variety. The loss ranged from 42·4 per cent when infection took place at the flowering stage to as much as 93·8 per cent when infection commenced at the seedling stage. In the case of the resistant variety also there was some loss in grain yield. The earlier the infection, the greater was the loss. The yield reduction was principally brought about by the production of fewer kernels per head and, to a lesser extent, by a reduction in the weight of the individual kernels. The average yield of total dry matter and straw was also considerably reduced in the case of the susceptible variety, though not to the extent of the grain yield. In the case of the resistant variety, the reduction was small at all stages and probably within the experimental error.

A striking effect of heavy rust infection in the case of the susceptible variety was the marked deterioration of the roots as indicated by discolouration, loss of weight and decrease in the number of fibrous roots. The groups that were infected late showed about the same amount of damage as those infected early in the season. In the case of the resistant variety, however, no deterioration was observed.

* Booting = the stage just before the ears emerge when they are still enclosed by the sheath.

Brown rust infection also caused a reduction in the number of heads and height of plant, prolonged the time required to reach full heading and maturity, and increased the water requirements of plants, in the case of the susceptible variety. Similar effects were noted in the resistant variety too but on a much smaller scale.

The interesting and important results of Johnston and Miller summarised above lead one to a consideration of the position with regard to brown rust of wheat in India. Whereas in the great wheat-growing regions of Canada and the United States of America, black rust appears to be the most important rust, in India brown rust and yellow rust are equally important. In fact in Bihar and the United Provinces where the growing period for wheat is comparatively short (November-March), experience indicates that black rust generally arrives too late in the season to be able to do much damage, and it is the brown and yellow rusts which are mainly responsible for the reduction in yield.

As in the case of the other two rusts, physiologic forms of brown rust are known to exist, that is to say, strains of the parasite exist which are morphologically indistinguishable from each other but which differ in their ability to infect different varieties of wheat. Over fifty physiologic forms of brown rust are known and the number is increasing as more work in various parts of the world is done. In India two physiologic forms—Form 10 and another—have been distinguished so far [Mehta, 1933]*. Of the Indian wheats tested, none is resistant to the first form but the newer Pusa varieties, Pusa 114, Pusa 120, Pusa 125, and Pusa 165, are highly resistant to the second form.† Physiologic form 9 with which Johnston and Miller worked has not so far been recorded in India.

The breeding of rust-resistant wheats for India has recently been taken up at Pusa and at a sub-station at Simla, in collaboration with Prof. K. C. Mehta of Agra and with the help of a grant from the Imperial Council of Agricultural Research. Until Prof. Mehta took up work on this subject, it was not known as to what physiologic forms of the three rusts existed in India and although some striking successes were obtained, notably at Pusa, previous breeding for rust resistance may be said to have been carried on in the dark. Now however a definite scientific procedure is possible. A large number of wheat varieties is being tested for resistance to the Indian physiologic forms of rust. As stated above, none of the Indian varieties yet tested is highly resistant to both the physiologic forms of brown rust occurring in India. Fortunately, however, a few foreign varieties are known to be resistant and these will be crossed with the best of the Indian wheats with the object of securing a wheat combining resistance to rust with other economically desirable qualities. It is hoped to obtain resistance not only to brown rust but also to the black and yellow rusts.

* K. C. Mehta, 1933. *Ind. J. Agric. Sci.* **3** : 939-62.

† K. C. Mehta—Unpublished data.

NOTICE

BIBLIOGRAPHY OF SOIL SCIENCE, FERTILIZERS AND GENERAL AGRONOMY

The Imperial Bureau of Soil Science has recently issued a complete " Bibliography of Soil Science, Fertilizers and General Agronomy " covering the whole scientific literature of the world on soil and allied sciences during 1931-1934. This extremely comprehensive bibliography gives the reference to practically every paper published during that period on the different branches of soil science, both pure and applied, on the use of fertilizers, on the cultivation of all the chief economic crops (over 140, including forests, are listed in the index) and on plant diseases in relation to soils. The references are arranged by subjects according to the Universal Decimal Classification, which is adequately explained in the Preface. The volume also contains an index to the Decimal Classification, an alphabetical cross-index to every subject on which the papers listed have been written, an author index containing over 4,000 names, and a list of the abbreviations used, and the full titles and places of issue (where known) of 800 journals, etc., from which the references in the Bibliography have been taken.

The Bibliography has been compiled from the references which appeared in the first 41 issues of the Bureau's monthly lists of " Publications relating to Soils and Fertilizers ". An unusual feature is that the literature of almost every country has been covered equally well, thanks to the exceptional facilities for procuring foreign periodicals which the Soil Bureau enjoys. Considering its scope and the amount of information it contains, the book has been made remarkably compact, without detriment to its readability. It should be a most valuable reference book to every agricultural scientist whose work is in any way connected with the soil. The Bureau intends to issue further similar bibliographies at intervals of 3-4 years, and in this way gradually to build up a complete reference library to the whole literature of the soil over an extended period of time.

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Index (post-free) Single part . . . . . 2*s.*

Title page and index . . . . . 3*s.*

Occasional Publication

Report on the Third Mycological Conference, 1934.

APPENDIX

IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH

INSTRUCTIONS TO AUTHORS OF PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuation should be done in the MS. and not left for introduction in proofs.

2. The title of a paper should not be lengthy.

3. It is desirable that the MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper roman numerals should be used. The use of arabic figures and (*a*), (*b*), (*c*), etc., is generally reserved for numbering the subdivisions coming under each head.

4. Articles submitted for publication either in the *Indian Journal of Agricultural Science* or in the *Indian Journal of Veterinary Science and Animal Husbandry* should be accompanied by abstracts for publication in *Agriculture and Live-stock in India*. Abstracts should be concise, but should be long enough to explain the matter dealt with ; ordinarily no abstract should exceed 200 words.

5. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in SM. CAP. with two lines, in CAPITALS with three lines, and when in **Antique** (heavy type) with a wavy line (~~~~~~~~~~).

6. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.

7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, *e.g.*, *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, *e.g.*, *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, *e.g.*, Striped Mauritius, Dharwar-American cotton and Broach cotton.

8. Botanical and zoological names are printed in italics and sh ould be underlined in the MS., *e.g.*, *Triticum vulgare* L. ; *Diplodia Corchori* Syd.; *Pyrilla aberrans* Kirby. The International Rules of Botanical Nomenclature and the

* Spare copies of these Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter ; they are printed in roman type (*e.g.*, calcium carbonate, prussic acid).

9. The following and similar abbreviations may be used freely :—*viz.*, *e.g.*, *i.e.*, mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c.c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer), ch. (chattack). Other abbreviations should be used sparingly, if at all.

10. References to plates should be given within brackets, without prefixing the word " see " or " cf.", in the MS. itself, and should not be left over for introduction in proofs. For example, " The parasite (Pl. X, fig. 4) was present late in 1906 ".

11. The word " Table " is preferable to " Statement ", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally ; for this purpose they should not exceed in width the printing measure of the page (5 in.). *Example*—

TABLE IV

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916-17

Station	No. of irrigations including the preliminary watering	Yield per acre in maunds and seers		Average yield per acre	
		Grain	Straw	Grain	Straw
		Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
Gungapur . . .	One	12 19½	20 10	9 34	21 17
Haripur . . .	,,	8 31	19 14		
Sargodha . . .	,,	8 12½	25 27½		

12. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be indicated by

the author's name followed by the year of publication enclosed in brackets ; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after the year both in the text and in the collected references. This system of referencing is the same as is used in the *Biochemical Journal* with slight modification and will be clear from the following illustration :—

The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921] who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

REFERENCES

Coward, K. H. and Drummond, J. C. (1921). *Biochem. J.* **15**, 530.
Osborne, T. B. and Mendel, L. B. (1919, 1). *J. Biol. Chem.* **37**, 187.
——————————(1919, 2). *J. Biol. Chem.* **41**, 549.
Steenbock, H. and Boutwell, R. (1919). *J. Biol. Chem.* **41**, 149.
Wilson, J. (1922). *J. Biol. Chem.* **51**, 455.

Abbreviations, as far as possible, should follow the system adopted in " A World List of Scientific Periodicals " published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognized symbols which will be found in the " Standard Dictionary " should be used. Second (page) proofs will be submitted to authors who should return them promptly.

Illustrations.

14. As the *format* of the journals has been standardized, the size adopted being crown quarto (about $7\frac{1}{8}'' \times 9\frac{5}{8}''$ cut), no text-figure, when printed, should exceed $4\frac{1}{2} \times 5$ inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being $5\frac{3}{4} \times 8$ inches exclusive of that for letterpress printing.

15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction linear measurements are understood ; thus, " half-size " means reduce to half the length and breadth, not half

the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required.

18. For three-colour work, copy may be oil-painting, water-colour, coloured photograph or coloured transparency, and larger than the size required. In preparing copy, one should use only the primary colours, in any combination, as only inks of primary colours are used in printing. Originals can be enlarged, if necessary, but this should be avoided if possible.

19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on "Photographic Illustrations" in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on "The Preparation and Reproduction of Scientific Illustrations" in the *Proceedings of the Third Entomological Meeting*, 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa.*

GIPD—S2—299 IC of AR—11-2-36—800.

ERRATA.

Page No. 146, Table IX, last line '*F*=below 0·01.' should be corrected to '*P*=below 0·01.'

Page 157, Table XVII, line 18 from bottom, columns 2 and 3 from right, the portion of the vertical line [between 'From ' and 'T80 (on '] should be deleted.

Page 188, line 5, comma (,) between 'M.A.' and '(Cantab.)' should be deleted.

Page 228, Table XIX, first column, last but one line, '21st Dec.' should be corrected into '1st Dec.'

Page 229, Table XIX, 1st column, 1st line, '23rd Dec.' should be corrected into '3rd Dec.'

Page 349, line 22, 'absorption' should be corrected into 'adsorption'.

Page 377, line 9 from bottom, insert comma (,) before 'humidity'.

Page 383, line 1, '*interna*' should be corrected into '*internal*'.

Page 391, line 33, 'patholocical' should be corrected into 'pathological'.

Page 401, Table II (*b*), column I, row 1, '$\sqrt{\frac{1}{2}\left(\frac{1}{n^1}+\frac{1}{n^2}\right)}$' should be corrected to '$\sqrt{\frac{1}{2}\left(\frac{1}{n^1}+\frac{1}{n^2}\right)}$'.

Page 403, Table VII, column 1, row 1, '*t*' should be inserted above '*P*'.

Date

ORIGINAL ARTICLES

STUDIES IN INDIAN PULSES

THE INHERITANCE OF MORPHOLOGICAL CHARACTERS AND OF WILT-RESISTANCE IN *RAHAR* (*CAJANUS INDICUS* SPRENG.)

BY

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(Received for publication on 21st December 1935)

(With Plates XXIII—XXVI)

INTRODUCTION

The property of disease resistance is generally a physiological quality which cannot be visibly identified by an observer in the same way as a morphological character. It may happen that a resistant type owes its resistant quality to some definite visible morphological character or that certain morphological characters, not in themselves directly concerned in the quality of resistance, are invariably present in a resistant type. Where this is the case the recognition of resistant individuals is easy.

In the case of the wilt disease of *rahar*, *Cajanus indicus* Spreng. (pigeon-pea), the existence of resistance in certain pure lines has been already demonstrated. It has been shown that a high degree of resistance is present in the Pusa Type 80 and that resistance is also possessed by Types 16, 41, 50, 51 and 82 [McRae and Shaw, 1933].

For the study of the inheritance of wilt-resistance it was necessary to select two types of *rahar* one of which possessed a high degree of resistance and another which was characterised by a marked susceptibility to the disease. Fortunately the Pusa Type 5 was all that could be desired in this latter respect, this type giving about 100 per cent deaths due to wilt in an artificially infected field. By growing the F_1 and F_2 generations of a cross between Type 5 and the resistant Type 80 in artificially infected fields the percentage loss due to wilt in these generations was determined.

The cross was grown in F_1, F_2 and F_3 generations both in fields which had been artificially infected and in fields which were not artificially infected. In this way by comparing the phenotypic frequencies in the surviving plants of the F_2 in the infected area with those in the F_2 grown under normal conditions it was possible to determine whether the quality of resistance was restricted to, or increased in, any particular phenotypes. The F_3 and subsequent generations grown under infected conditions as well as under normal conditions served to elucidate the inheritance of wilt resistance.

Part I

The inheritance of morphological characters—characters of parents and F_1

The characters of the parents have already been described and are shown with those of the F_1 in the following table :—

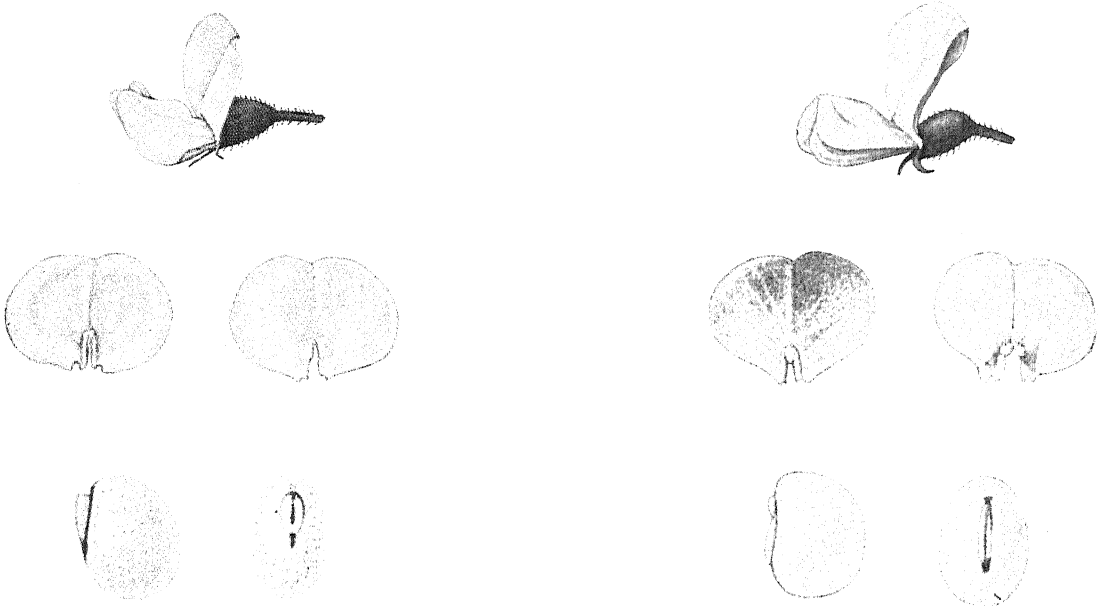
Table I

Characters of parents and of F_1

Type	Habit	Inflorescence	Flower	Pod	Seed
Type 80 .	Spreading and tall.	Open .	Yellow with diffused red colour on dorsal side of standard	Green with black streaks	Light fawn with brown markings (patches)
Type 5 .	Erect and short	Crowded	Yellow . .	Green with black streaks.	Silver-white with faint grey dots.
F_1—Type 80 × 5; Type 5 × 80	Erect (Intermediate) and short.	Crowded	Yellow with faint red veins on dorsal side of standard	Green with black streaks	Pinkish silver-white with brown markings (patches)

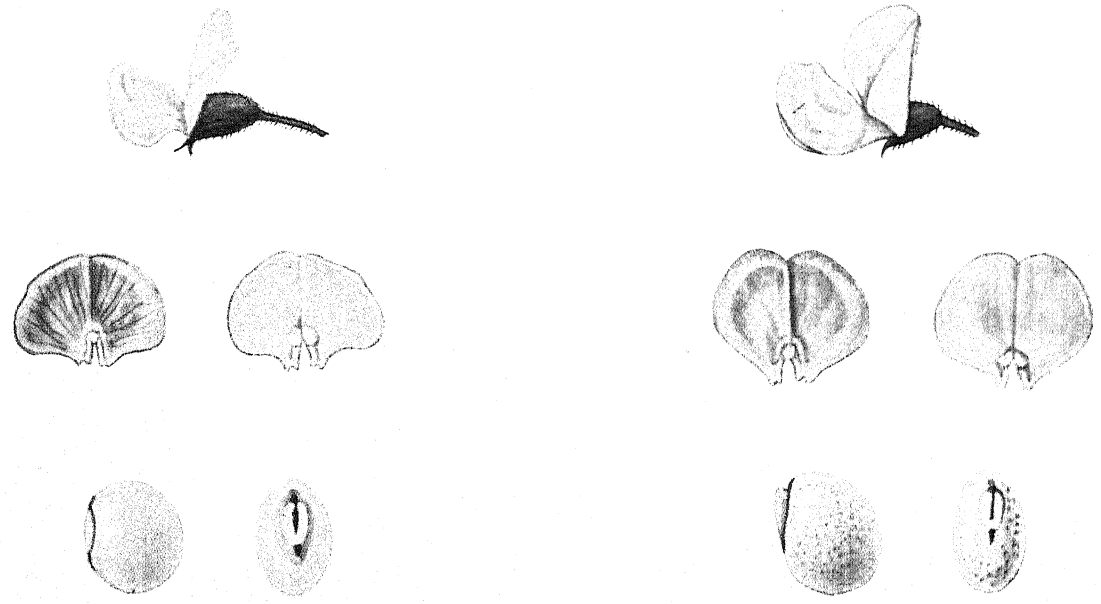
The characters selected for study were those relating to the habit, inflorescence, flower and seed. In F_1 the erect and short habit of Type 5 was dominant over the tall and spreading habit of Type 80, the erectness being partially dominant.

FLOWER AND SEED COLOURS IN PIGEON-PEAS – TYPE 5 × TYPE 80 – F_2



1
Type 5 parent

2
Type 80 parent



3
Type 5 × Type 80 — F_1

4
Ringed flower and Type 1 seed

Similarly the crowded inflorescence of Type 5 was dominant over the open inflorescence of Type 80. The seed of the F_1 resembled that of Type 5 in having silver-white background but possessed faint brown markings like that of Type 80. The flower of F_1, however, was a new phenotype unlike either parent, possessing red colour restricted to the veins on the dorsal side of the standard.

Crossing was easy when Type 80 was the female parent but was more difficult when Type 5 was the female parent.

The F_2 generation

It is convenient to describe first the inheritance of characters in this generation in the population which was grown in a non-infected field. The frequencies of phenotypes in this normal population can then be contrasted with those in the infected area.

(*1*) *Flower colour*

The F_1 had a yellow flower with faint red veins on the dorsal side of the standard. In F_2 both parental types and the F_1 type were realised and a fourth new phenotype appeared as the recessive (Plate XXIII). This latter type is called "ringed" and is characterised by the possession of red dots on the margin of the dorsal side of the standard, in other respects this flower resembled that of Type 5. The frequencies of these phenotypes in F_2 are shown in Table II.

Table II

Segregation in F_2 for flower colour

	F_1	Type 5	Type 80	Ringed	Total
Type 80 × Type 5.	Observed 457 .	152	146	59	814
	Expected 458·1 . (9 : 3 : 3 : 1)	152·7	152·7	50·9	814·4
			$\chi^2=1·6$,	$P=0·66$	
Type 5 × Type 80.	Observed 487 .	164	180	53	884
	Expected 497·25. (9 : 3 : 3 : 1)	165·75	165·75	55·25	884
			$\chi^2=1·5$,	$P=0·67$	

A dihybrid segregation, 9 : 3 : 3 : 1, is indicated and the fit is close, $P=0·66$

(2) Inflorescence

Type 5 has a " crowded " inflorescence, with short internodes and flowers in clusters, and Type 80 has an open inflorescence. In F_1 the inflorescence was crowded (Plate XXIV, fig. 1) and in F_2 the following segregation was observed :—

TABLE III

Segregation in F_2 for inflorescence character

		Crowded	Open	Total
Type 80 × Type 5	Observed	609	211	820
	Expected (3 : 1)	615	205	820
	Deviation = 6	S. E. = 12·32		
	$\frac{\text{Dev.}}{\text{S.E.}} = \frac{6}{12 \cdot 32} = 0 \cdot 49$			
Type 5 × Type 80	Observed	661	222	883
	Expected (3 : 1)	662·25	220·75	883
	Deviation = 1·25	S. E. = 12·87		
	$\frac{\text{Dev.}}{\text{S.E.}} = \frac{1 \cdot 25}{12 \cdot 87} = 0 \cdot 097$			

The ' crowded ' character is dominant in F_1 and F_2 segregation takes place on a 3 : 1 ratio.

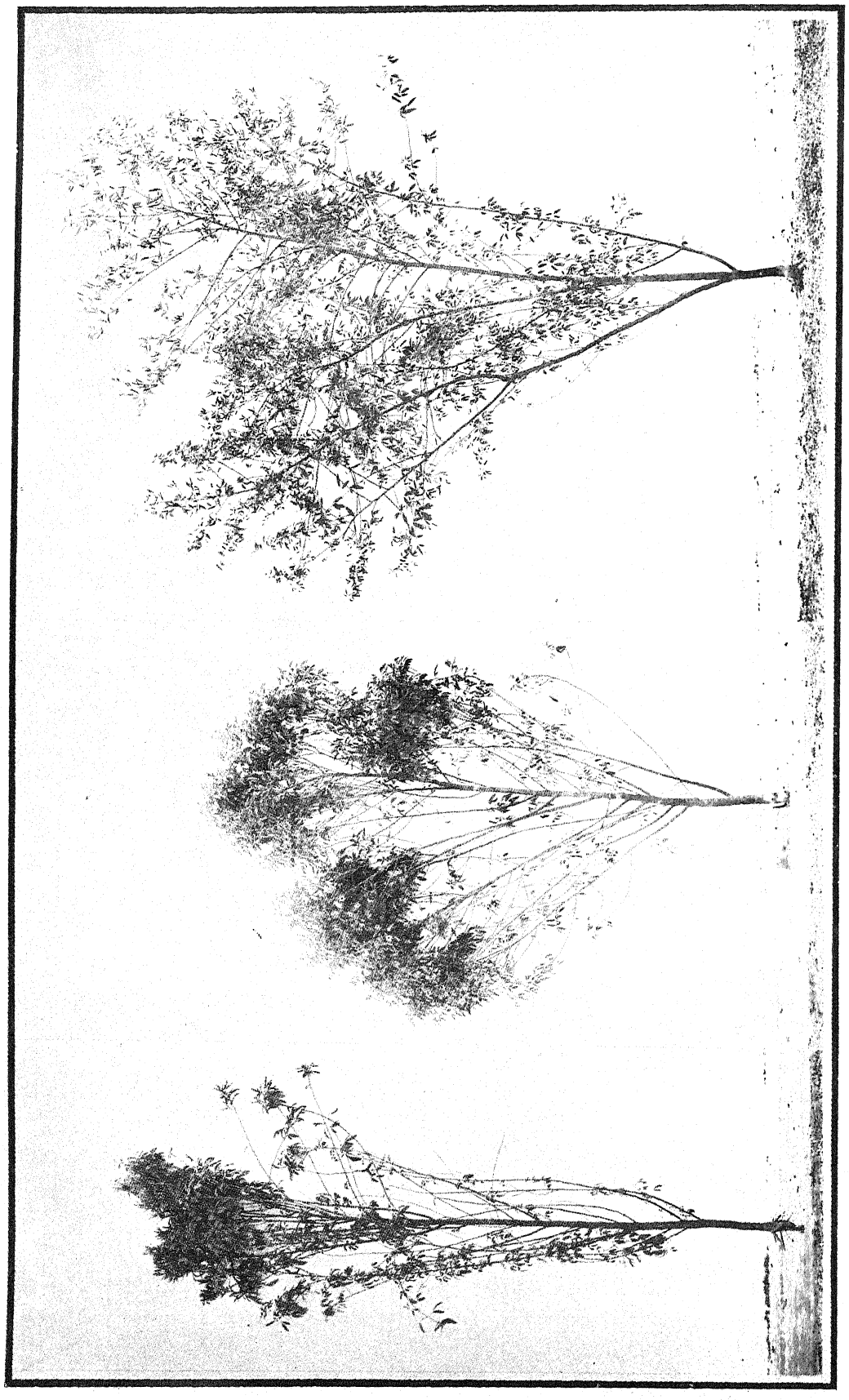
T. 5 F_1 T. 80

FIG. 1 Types of inflorescence

a *b* *c* *d*

FIG. 2 Plant habit and types of inflorescence :

a. Erect plant with crowded inflorescence.
b. Erect plant with open inflorescence.
c. Spreading plant with open inflorescence
d. Spreading plants with crowded inflo



Plant habit (Type 80 × Type 5)

(3) *Habit*

The plant habit of Type 5 was erect, that of Type 80 was spreading and in F_1 the erect habit showed incomplete dominance, the plants of F_1 being less erect than Type 5. It was impossible however in F_2 to separate with any certainty the F_1 and Type 5 phenotypes ; they are therefore classified together.

TABLE IV

Segregation in F_2 for habit

		Erect and Inter-mediate	Spreading	Total
Type 80 × Type 5	Observed	601	231	832
	Expected (3 : 1)	624	208	832
	Deviation = 23	S. E. = 12·49		
	$\frac{\text{Dev.}}{\text{S. E.}} = \frac{23}{12 \cdot 49} = 1 \cdot 84$			
Type 5 × Type 80	Observed	644	217	861
	Expected (3 : 1)	645·75	215·25	861
	Deviation = 1·75	S. E. = 12·70		
	$\frac{\text{Dev.}}{\text{S. E.}} = \frac{1 \cdot 75}{12 \cdot 70} = 0 \cdot 14$			

A single-factor difference for plant habit is indicated. The classification of phetontypes is somewhat difficult as the habit of the plant is influenced by the spacing in the field.

(4) *Stature*

In regard to stature, in the experiment of 1929-30, Type 5 had a mean height of 251 cm. with a range of 220-275 cm. Type 80 had a mean height of 320 cm. with a range of 285-345 cm. F_1 (T. 5 × T. 80) showed a mean of 255 cm. and F_1 (T. 80 × T. 5) a mean of 270 cm. The F_2 had a mean of 276 cm. with a range of 205-375 cm. The similarity of the mean and range of the frequency distribution in F_1 with that of Type 5, the shorter parent (Table VII) suggests that

short stature is dominant and indeed if we take a height of 290 cm. as the upper limit of dwarfness we can classify F_2 thus :—

TABLE V

Segregation in F_2 for stature of plants in 1929-30

		Short	Tall	Total
Type 80 × Type 5	Observed	561	214	775
	Expected (3 : 1)	581·25	193·75	775

Dev. = 20·25 S. E. 12·05

$$\frac{\text{Dev.}}{\text{S. E.}} = \frac{20 \cdot 25}{12 \cdot 05} = 1 \cdot 68$$

The reciprocal cross (Type 5 × Type 80) was grown again two years later (1932-33) and in this season both parents grew taller, the conditions for growth being more favourable than in the previous season. The mean of Type 5 was 285 cm. with a range of 255-315 cm. and the mean of Type 80 was 352 cm. with a range of 305-375 cm. The mean of F_1 was 290 cm. with a range of 215-325 cm. The mean of F_2 in this year was 322 cm. with a range of 215-395 cm. The mean of F_2 in 1932-33 was therefore 46 cm. higher than in the earlier season and we have taken the upper limit of dwarfness in this season as 340 cm., *i.e.*, 50 cm. above the limit of 290 cm. used in the cross, Type 80 × Type 5.

Classifying the F_2 of the cross, Type 5 × Type 80 on this basis we obtain—

TABLE VI

Segregation in F_2 for stature in 1932-33

		Short	Tall	Total
Type 5 × Type 80	Observed	670	206	876
	Expected (3 : 1)	657	219	876

Dev. = 13 S. E. = 12·82

$$\frac{\text{Dev.}}{\text{S. E.}} = \frac{13}{12.82} = 1 \cdot 014$$

An examination of the F_2 frequency arrays, however, does not support this hypotheis of a monofactorial difference between tall and short. The F_2 arrays are fairly smooth curves and show no evidence of a natural division at the points at which demarcation should occur on this theory, but the evidence of linkage and the segregations in F_3 tend to support the monofactorial theory of the inheritance of height.

TABLE VII

Frequency distribution of plant height

Name of culture	Year	Plot	Height in centimeters																				Population	Mean	S. E. (N)	σ
			205	215	225	235	245	255	265	275	285	295	305	315	325	335	345	355	365	375	385	395				
Type 5 parent	1929-30	Orchard 4B			4	2	2	7	7	1													23	251·69	3·21	15·26
Type 80 parent	1929-30	Orchard 4B									1	2	2	2	3	3	3						16	320·62	4·72	18·70
(80 × 5) F_1	1929-30	Orchard 4B				1	2	5	1	2													11	255·91	3·55	11·64
(5 × 80) F_1 .	1929-30	Orchard 4B				1	1	2	1	7	4												16	270·00	3·69	14·58
(80 × 5) F_2 .	1929-30	Orchard 4B	4	4	17	45	76	101	132	107	75	44	29	37	46	28	21	4	3	2	...	...	775	276·85	1·14	31·46
(5 × 80) F_2 .	1932-33	N. T. G. 3	...	3	1	5	2	7	8	17	26	66	118	157	168	92	71	50	41	32	11	1	876	322·70	0·93	27·04
Type 5 parent	1932-33	N.T.G. 3						1	5	12	26	19	3	1									67	285·45	1·35	10·98
Type 80 parent	1932-33	N.T.G. 3											1	1	1	5	8	10	15	3			44	352·95	2·34	15·31
F_1	1932-33	N.T.G. 3			1	...	2	1	1	1	2	1	2	4	3								18	290·00	6·36	30·78

If we adopt the monofactorial theory for the inheritance of stature and the two characters of inflorescence and stature are considered together we observe :—

TABLE VIII

Relation of stature and nature of inflorescence in F_2 : Cross Type 80 × Type 5 (1929-30)

	Short Crowded	Tall Crowded	Short Open	Tall Open
Observed	534	36	27	178
Expected on 9 : 3 : 3 : 1 . . .	435·6	145·2	145·2	48·4

$\chi^2 = 547 \cdot 624$, P = below 0·01

Similarly in the reciprocal cross we observe :—

TABLE IX

Relation of stature and nature of inflorescence in F_2 : Cross Type 5 × Type 80 (1932-33)

	Short Crowded	Tall Crowded	Short Open	Tall Open
Observed	629	33	41	173
Expected	492·75	164·25	164·25	54·75

$\chi^2 = 490 \cdot 41$, F = below 0·01

Taken separately both characters show a 3 : 1 ratio but combined they depart widely from the expected dihybrid ratio, the frequencies of the parental phenotypes being much increased. Linkage between the short and crowded characters is indicated, with a cross-over value of about 8·5 per cent in Table VIII, and of about 8·8 per cent in Table IX.

Plate XXIV, fig. 2 illustrates that plants with crowded type of inflorescence, whether erect or spreading, are shorter than the plants with the open type of inflorescence.

5. Seed colour

The seed of Type 5 is silver-white with faint grey markings and that of Type 80 is fawn with brown markings. The seed of F_1 is pinkish silver-white with brown markings (Plate XXIII). The brown markings are therefore dominant and in F_2 we observe :—

TABLE X

Segregation in F_2 for seed colour

		Seed with brown markings like F_1 or Type 80	Seed like Type 5	Total	Significance
Type 80 × Type 5	Observed	578	198	776	Dev. = 4·0 S. E. = 12·06
	Expected (3 : 1)	582	194	776	$\frac{\text{Dev.}}{\text{S. E.}} = 0{\cdot}33$
Type 5 × Type 80	Observed	661	207	868	Dev. = 10·0 S. E. = 12·76
	Expected (3 : 1)	651	217	..	$\frac{\text{Dev.}}{\text{S. E.}} = 0{\cdot}78$

Owing to the wide range of variation in the phenotype with brown markings it was impossible to distinguish with certainty in F_2 the seed phenotype of Type 80 from that of F_1, the Type 5 phenotype, however, could always be clearly recognised. We have, therefore, indicated a monohybrid ratio for seed colour although we strongly suspect that the inheritance of this character is more complicated. Even if the inheritance of seed colour is proved at some future date to be more complicated the main segregation (3 : 1) between Type 5 and others will remain valid and any further elucidation of the position will simply show the type of inheritance of other factors within this framework. Flower colour and seed colour show linkage, brown markings on the seed being linked with the F_1 and Type 80 phenotypes for flower colour. If we classify the F_1 and Type 80 phenotypes

together as 'coloured' flowers and the Type 5 and 'Ringed' phenotypes as 'non-coloured' we obtain:—

TABLE XI

Relation of flower and seed colours in F_2

	Coloured flower and coloured seed	Coloured flower and white seed	Non-coloured flower and coloured seed	Non-coloured flower and white seed
Type 80 × Type 5 . .	575	4	3	194
Type 5 × Type 80 . .	652	4	9	203

The cross, Type 80 × Type 5 shows a cross-over value of 0·9 per cent and the reciprocal a value of 1·52 per cent. The evidence of F_3, however, indicates that the linkage is absolute and that seed and flower colour are determined by the same factor or factors.

Theory

We may suppose—

A=factor for red in standard and for brown spotting on seed coat.

B=factor restricting red colour to veins in the standard in the presence of **A** and inibiting red dots in the absence of **A**.

P=factor for erect habit

S=factor for short stature

K=factor for crowded pod habit

Type 5=**aB (K S) P.**

Type 80=**Ab (k s) p.**

In flower colour in F_2 we are dealing with a segregation of **A** and **B** which will give—

9 AB=F_1 flower with red veins on dorsal side of standard

3 Ab=Type 80 flower with red colour diffused over standard

3 aB=Type 5 flower—yellow

1 ab='ringed flower'

These phenotypes are all realised in the above proportions in F_2.

In seed colour we have a segregation of—

AA=brown markings like Type 80 and fawn background

Aa=faint brown markings and pinkish silver-white background

aa=silver-white like Type 5.

THE F_3 GENERATION

(1) *Flower colour*

The behaviour of cultures in F_3 as regards flower colour is shown in the table below :—

TABLE XII

F_3 behaviour for the inheritance of flower colour

No. of F_3 cultures and nature of their parents in F_2	Flower colour segregation in F_3	Formulae	Frequencies		Seed colour segregation in F_3
			Observed	Expected	
32 F_1 .	Pure F_1 . .	1 **AABBCC**	2	3·55	Pure "brown markings"
	3 F_1 : one Type 80	2 **AABbCC**	9	7·1	Do.
	3 F_1 : one Type 5.	2 **AaBBCC**	5	7·1	3 Brown markings : one silver-white
	9 F_1 : 3 type 80 . 3 Type 5 : one Ringed	4 **AaBbCC**	16	14·2	Do.
14 Type 80 .	Pure Type 80 .	1 **AAbbCC**	5	4·66	Pure brown markings
	3 Type 80 : one Ringed	2 **AabbCC**	9	9·33	3 Brown markings : one silver-white
9 Type 5 .	Pure Type 5 .	1 **aaBBCC**	1	3·0	Pure Type 5 (silver-white)
	3 Type 5 : one Ringed	2 **aaBbCC**	8	6·0	Do.
5 Ringed .	Pure ringed .	1 **aabbCC**	5	5	Do.

Segregation in the F_1 group splitting into 3 F_1 : 1 Type 80 :—

	F_1	Type 80	Total
Observed	471	179	=650
Expected	487·5	162·5	
Deviation	16·5	16·5	

S. E. = 11·04

$$\frac{\text{Dev.}}{\text{S. E.}} = \frac{16\cdot5}{11\cdot04} = 1\cdot49.$$ The fit is good.

Segregation in the F_1 group splitting into 3 F_1 : one Type 5 :—

	F_1	Type 5	Total
Observed	237	90	=327
Expected	245·25	81·75	
Deviation	8·25	8·25	

S. E. = 7·83

$$\frac{\text{Dev.}}{\text{S. E.}} = \frac{8\cdot25}{7\cdot83} = 1\cdot05.$$ The fit is good.

Segregation in the F_1 group splitting into 9 F_1 : 3 Type 80 : 3 Type 5 : one Ringed :—

	F_1 :	Type 80 :	Type 5 :	Ringed	Total
Observed . .	610	204	202	73	=1089
Expected . .	612·5	204·2	204·2	68·1	
Deviation . .	2·5	0·2	2·2	4·9	

$\chi^2 = 0\cdot386$

P—Between 0·90 and 0·95. The fit is good.

Segregation of the splitting Type 80 :—

	Type 80 :	Ringed	Total
Observed	462	149	=611
Expected	458·25	152·75	
Deviation	3·75	3·75	

S. E. = 10·70

$$\frac{\text{Dev.}}{\text{S. E.}} = \frac{3\cdot75}{10\cdot70} = 0\cdot35.$$ The fit is good.

Segregation of the splitting Type 5 group :—

	Type 5 :	Ringed	Total
Observed	405	160	=565
Expected	423·75	141·25	
Deviation	18·75	18·75	

S. E. = 10·29

$$\frac{\text{Dev.}}{\text{S. E.}} = \frac{18\cdot75}{10\cdot29} = 1\cdot82.$$ The fit is good.

The nature of the segregations in F_3 and their respective frequencies confirm the dihybrid theory for flower colour. The segregation for seed colour is also shown in Table XII and agrees with the theory previously set out.

(2) *Inflorescence*

TABLE XIII

F_3 behaviour for the inheritance of inflorescence character

No. of F_3 cultures and nature of their parents in F_2	Segregation	Formulae	Frequencies	
			Observed	Expected
44 Crowded . .	Pure crowded . .	**KK**	14	14·66
	3 crowded : 1 open .	**Kk**	30	29·33
16 Open . .	Pure open . .	**kk**	16	16

The segregation in the spliting crowded F_2 plants was :—

	Crowded :	Open	Total
Observed	1586	504	=2090
Expected (3 : 1)	1567·5	522·5	
Deviation	18·5	18·5	

S. E. = 19·80

$$\frac{\text{Dev.}}{\text{S. E.}} = \frac{18\cdot50}{19\cdot80} = \mathbf{0\cdot93}.$$ The fit is good.

The dominance of the 'crowded' habit and its inheritance on a 3 : 1 ratio are confirmed.

(3) *Habit*

Erect and spreading.—Owing to the wide range of variation in the spreading, intermediate and erect phenotypes the observed segregation in F_3 cultures revealed several instances of wrong diagnosis in F_2, but the general result of F_3 confirms our theory as shown in the following table :—

TABLE XIV

F_3 behaviour for the inheritance of habit

No. of F_3 cultures and nature of their parents in F_2	Segregation	Formulae	Frequencies	
			Observed	Expected
15 Erect .	Pure erect . . .	**PP**	15	15
30 Intermediate	1 Erect : 2 Intermediate : 1 Spreading	**Pp**	26	30
	Pure erect . . .	**PP**	1	*Nil*
	Pure spreading . .	**pp**	3	*Nil*
15 Spreading .	Pure spreading . .	**pp**	12	15
	1 Erect : 2 Intermediate : 1 Spreading	**Pp**	3	*Nil*

Thus in F_2 it is evident that one erect and three spreading plants were wrongly included among thirty intermediates and that one intermediate was wrongly classed among fifteen spreading individuals.

The total segregation in the twenty-six splitting intermediates is as follows :—

	Observed	Calculated on 1 : 2 : 1 ratio
Erects	447	434·25
Intermediates	888	868·50
Spreading	402	434·25
Total .	1737	

$\chi^2 = 3 \cdot 22$

P—between 0·1 and 0·2

4. Stature

In F_3 forty cultures were grown from plants which had been classed as 'short' in F_2 and twenty cultures from plants which had been classed as 'tall'. The nature of the segregations is summarised below :—

TABLE XV

F_3 behaviour of the inheritance of stature

No. of F_3 cultures and nature of their parents in F_2	Segregations in F_3	Frequencies	
		Observed	Expected
40 Short . . .	Pure short	13	13·3
	Segregating	26	26·6
	Pure tall	1	*Nil*
20 Tall . . .	Pure tall	15	20
	Segregating	4	*Nil*
	Pure short	1	*Nil*

The occurrence of segregating cultures in F_3 from parents which were classed as the double recessive tall phenotype in F_2 is to be expected as some cases of wrong diagnosis must occur in classifying a quantitative character such as this in which there is an overlap between the upper and lower limits of the two phenotypes and in which variations in soil fertility will have a marked influence on the development of phenotypes.

The F_2 generation showed strong linkage between short stature (factor **S**) and crowded inflorescence (factor **K**). The cross-over value was calculated at 8·5 per cent and on this basis we conclude that the F_1 produced gametes in the

ratio of 45·75 **SK** : 4·25 **Sk** : 4·25 **sK** : 45·75 **sk**. The F_2 from these gametes should form the different genotypes as noted below :—

Genotype	Frequencies		Remarks
SSKK	2093·06	Crowded short 7093·06	The ratio of crowded to open is 3 : 1 and similarly of short plants to talls is 3 : 1. The ratio of the four phenotypes is about 17·3 : 1 : 1 : 5·8 which agrees fairly closely with the observation.
SsKK	388·88		
SSKk	388·88		
SsKk	4222·24		
ssKK	18·06	Crowded tall 406·94	
ssKk	388·88		
SSkk	18·06	Open short 406·94	
Sskk	388·88		
sskk	2093·06	Open tall 2093·06	
	10,000·00		

In our F_3 population of sixty cultures the observed and expected values were as shown in Table XVI below.

TABLE XVI

Relationship of habit and stature in F_3

No. of F_3 cultures and nature of parents in F_2	Segregation in F_3					
	Habit			Stature		
	Nature of segregation	Frequencies		Nature of segregation	Frequencies	
		Observed	Expected		Observed	Expected
39 crowded short plants	Pure crowded	13	13	Pure short	12	11
				3 short : one tall	1	2
	3 crowded : one open	26	26	Pure short	1	2·2
				3 short : one tall	25	23·8
15 open tall	Pure open	15	15	Pure tall	15	15
5 crowded tall	Pure crowded	1	1·67	Pure tall	*Nil*	5
	3 crowded : one open	4	3·33	Pure short	1	*Nil*
				3 short : one tall	4	*Nil*
1 open short	Pure open	1	1	Pure tall	1	*Nil*

It will be observed that in the cross-over classes 'crowded, tall' and 'open, short' the behaviour in F_3 shows that the parent was wrongly diagnosed in F_2. Does this really mean that the linkage is absolute and that the supposed cross-overs are in all cases due to wrong diagnosis of stature ?

In Table XVII the five plants Nos. 51, 473, 413, 582 and 141 classed as 'crowded, tall' in F_2 had heights of 341, 342, 344, 344 and 357 cm. respectively, and the first four heights are very close to the upper limit of dwarfness. The one plant No. 715 classed in "open, short" had a height of 326 cm.—this should place it definitely among shorts. Table XVII shows the relationship of the nature of inflorescence and plant height in the F_3 cultures. It will be evident that the average height in cultures pure for crowded inflorescence is less than that in cultures which are pure for open type of inflorescence. Similarly in Table XVIII the distribution of plants with crowded inflorescence has definitely a lower range for stature than plants in the same culture which possessed the open type of inflorescence.

TABLE

Relation of nature of inflorescence and

Nature of inflorescence	Culture No.	Height of the parent plant in F_2	Frequency distribution of plant height													
			155	165	175	185	195	205	215	225	235	245	255	265	275	285
Pure crowded	T. 5 parent on the east side of F_3		1	6	12	12	9	2	4	6	4	2	5	2	...	...
Do.	F_3 No. 46	316 short	1	5	5	7	11	5	5	4	5	2	2	3	3	...
Do.	„ 99	324 „		1	1	3	14	6	10	10	7	...	6	2	1	...
Do.	„ 139	327 „				1	12	11	16	7	2	4	...	...	...	1
Do.	„ 186	322 „			1	1	12	11	15	10	3	2	1	1	...	...
Do.	„ 199	325 „					1	3	13	14	11	11	11	4	1	...
Do.	„ 261	328 „					4	9	6	10	7	8	8	4	2	...
Do.	„ 266	322 „				2	2	7	17	10	10	11	3	4	...	...
Do.	„ 405	329 „				1	1	3	6	14	18	13	6	1	2	...
Do.	„ 533	317 „			1	2	7	3	8	15	16	12	4	1	...	...
Do.	„ 580	314 „				6	6	8	22	19	10	...	...	...	...	...
Do.	„ 582	344 Tall		1	...	...	2	2	6	12	18	19	11	2	1	...
Do.	„ 627	339 Short					2	1	2	2	12	23	15	10	1	...
Do.	„ 670	329 „					1	...	13	...	24	10	7	2	...	...
Do.	„ 914	304 „							2	6	9	16	12	14	7	2
Do.	T. 5 (on the west side of F_3)						1	1	2	4	14	14	10	9	...	...
Pure open	T. 80 (on the east side of F_3)							3	5	7	9	5	8	3	5	6
Do.	F_3 No. 143	366 Tall							3	4	9	4	7	11	9	
Do.	„ 222	350 „								3	9	10	8	5	7	6
Do.	„ 268	345 „						1	—	2	9	10	7	6	9	15
Do.	„ 269	345 „								3	6	4	12	5	12	10
Do.	„ 319	349 „						3	4	2	4	7	6	5	14	5
Do.	„ 414	341 „									2	1	12	7	12	11
Do.	„ 475	385 „							1	1	2	7	7	9	17	7
Do.	„ 679	380 „									1	3	6	11	13	23
Do.	„ 715	326 Short									3	3	8	11	10	
Do.	„ 722	348 Tall								1	1	2	10	14	16	10
Do.	„ 723	363 „									1	...	6	4	11	13
Do.	„ 858	352 „									2	3	5	6	13	11
Do.	„ 917	378 „							1	...	...	1	3	9	14	14
Do.	„ 962	378 „										2	4	9	15	9
Do.	„ 963	351 „											1	2	11	8
Do.	„ 1007	360 „											2	3	6	9
Do.	T. 80 (on the west of F_3)												2	5	10	19

XVII

plant height in F_3 (T. 5 × T. 80).

in cm.					Total population	Average height in cm.	σ	C. V.	Lines away from parent line on the east	Calculated height of the parent cm.	Remarks
295	305	315	325	335							
...	...	...	...	...	65	201·1±3·72	29·7	14·7	0		Lines of cultures run north to south, and are 4 ft. apart from one another. There is a fertility gradient from east to west, the western side being richer than the eastern. This is shown by the increase in height in both the parents, on the western side, the parental rows having been put both on the eastern and western sides of the F_3.
...	...	...	...	...	58	208·4±4·29	32·5	15·6	1		
...	...	...	...	...	61	216·9±3·06	23·8	10·9	3		
...	...	...	...	...	54	213·5±2·30	16·7	7·8	4		
...	...	...	...	...	57	213·1±2·36	17·6	8·2	9		
...	...	...	...	...	69	234·4±2·27	18·7	7·9	10	208 T. 5	
...	...	...	...	...	58	231·4±2·99	22·6	9·7	12		T. 5 T. 80
...	...	...	...	...	66	226·8±2·31	18·7	8·2	14		Height on the east side . .. . 201·1 246·7
...	...	...	...	...	65	233·8±2·01	16·1	6·8	27	219 T. 5	Height on the west side . . 243·3 288·8
...	...	...	...	...	69	226·2±2·39	19·7	8·7	39	228 T. 5	Increase in height . 42·2 42·1
...	...	...	...	...	71	215·1±1·71	14·3	6·6	41		
...	...	...	...	...	74	235·8±2·03	17·3	7·3	42		
..	...	...	...	...	68	245·3±1·92	15·8	6·4	43		
..	...	...	...	...	57	235·0±1·58	14·8	6·3	45	232 T. 5	
...	...	...	...	...	68	251·2±2·07	16·9	6·8	53	238 T. 5	Segregating for height.
...	...	...	...	...	55	243·3±2·15	15·7	6·4	62		
...	1	...	...	...	52	246·7±3·48	25·0	10·1	From east)	T. 80 (on	
6	2	3	1	...	64	263·9±3·39	27·1	10·2	7	251 T. 80	
8	5	1	2	...	64	267·0±3·35	26·6	9·9	12		
9	...	...	...	...	68	264·7±2·78	22·6	8·5	17	258 T. 80	
8	3	3	...	...	66	270·0±2·90	23·4	8·7	18		
11	6	1	...	...	68	266·2±3·80	31·0	11·6	24	262 T. 80	
7	6	1	...	...	59	275·8±2·48	18·7	6·8	32		
13	...	5	...	1	70	274·4±2·78	23·0	8·3	36	271 T. 80	
13	5	...	...	...	75	278·7±1·86	15·9	5·7	47	278 T. 80	
8	5	1	...	...	56	273·9±2·69	19·7	7·2	48		
8	3	2	...	...	67	274·1±2·21	17·9	6·5	49		
13	7	5	3	...	63	286·9±2·54	19·4	6·7	50		
14	8	1	...	...	63	280·4±2·37	18·7	6·6	52		
17	10	...	...	...	69	282·8±2·06	16 9	5·9	55	284 T. 80	
9	8	8	...	...	64	284·3±2·42	19·2	6·7	57		
10	9	5	3	...	49	292·5±2·06	16·9	5·8	58		
13	11	11	4	1	60	296·8±2·37	18·2	6·1	61		
16	12	4			68	288·8±1·74	14·2	4·9	62		

TABLE

Plant height in the F_3 cultures that are segre-

Culture No.	Height of the parent plant in F_2 cm.	Frequency distribution of height in															
		155	165	175	185	195	205	215	225	235	245	255	265	275	285	295	305
T. 5 parent on the east side of F_2.		1	6	12	12	9	2	4	6	4	2	5	2	...	...	...	...
T. 80 parent on the east side of F_2.		...	...	...	...	...	3	5	7	9	5	8	3	5	6	...	1
F_2 No. 51 .	341 Tall . .	...	...	1	5	14	9	9	8	8	4	9	2	4	...	2	1
,, 141 .	357 ,, . .	...	...	1	...	1	4	4	15	14	6	7	4	3	...	3	2
,, 148 .	332 Short .	...	...	...	...	2	3	7	15	9	8	8	11	3	4	1	1
,, 185 .	332 ,, .	...	...	...	...	2	6	7	12	12	10	2	8	4	4	2	...
,, 263 .	318 ,, .	...	...	...	...	5	7	6	12	13	11	7	6	1	...	2	1
,, 267 .	299 ,, .	...	...	...	...	1	2	12	12	11	6	4	6	4	5	2	1
,, 272 .	317 ,, .	...	...	...	3	...	3	4	6	12	10	10	4	4	8	2	2
,, 308 .	323 ,, .	...	...	...	...	...	3	9	12	12	10	7	8	2	1	6	2
,, 310 .	304 ,, .	...	...	...	...	1	5	9	9	12	5	7	2	2	1	5	1
,, 311 .	329 ,, .	...	...	...	...	1	2	6	10	13	9	9	4	6	1	1	...
,, 314 .	315 ,, .	...	...	...	1	1	2	5	6	14	13	5	4	2	4	3	...
,, 355 .	305 ,, .	...	...	...	...	4	8	10	9	9	7	5	7	3	3	3	...
,, 365 .	320 ,, .	...	...	...	...	3	3	5	14	11	11	4	6	...	1	...	2
,, 366 .	334 ,, .	...	...	...	1	3	3	6	16	18	8	6	4	1	2	1	...
,, 410 .	324 ,, .	...	...	...	...	4	2	6	11	14	11	6	1	2	3	4	3
,, 412 .	337 ,, .	...	...	...	...	...	3	7	17	11	20	5	2	6	2	2	2
,, 413 .	344 Tall . .	...	...	...	...	1	1	2	8	6	13	17	9	4	1	4	3
,, 436 .	323 Short .	...	...	2	1	4	6	7	7	10	13	4	9	2	2	4	...
,, 437 .	322 ,, .	...	...	...	...	2	3	3	7	9	17	6	3	2	9	4	1
,, 473 .	342 Tall . .	...	...	...	...	2	1	6	9	6	17	14	3	4	4	2	1
,, 476 .	333 Short .	...	...	2	...	1	7	7	7	13	12	11	1	7	...	1	...
,, 478 .	308 ,, .	...	...	...	3	4	4	18	12	1	7	6	1	1	1	2	1
,, 485 .	327 ,, .	...	...	...	...	4	4	4	13	9	6	5	2	...	...	...	...
,, 574 .	318 ,, .	...	...	2	...	2	4	9	13	21	11	2	2	3	1	...	...
,, 669 .	336 ,, .	...	...	...	...	...	2	1	3	11	20	15	7	4	3	3	...
,, 724 .	327 ,, .	...	...	...	...	...	...	...	...	10	20	10	12	7	5	3	1
,, 908 .	312 ,, .	...	...	...	...	...	2	1	3	6	9	10	12	15	4	...	...
,, 918 .	322 ,, .	...	...	...	...	...	...	4	1	12	7	17	11	10	2	4	...
,, 964 .	327 ,, .	...	...	...	...	2	...	3	...	11	14	10	15	9	5	3	...
,, 1006 .	322 ,, .	...	...	...	...	2	2	...	7	3	9	20	13	6	7	1	...
T. 5 on the west side of F_2		...	...	...	...	1	1	2	4	14	14	10	9	...	...	...	...
T. 80 on the west side of F_2.		...	...	...	...	...	...	...	...	...	...	2	5	10	19	16	12

XVII—*contd.*

gating for the nature of inflorescence

cm.				Total population	Average	σ	C. V.	Lines away from the parents	Remarks
315	325	335	345						
...	...	...	...	65	201·1±3·72	29·7	14·7		
...	...	...	...	52	246·7±3·48	25·0	10·1		
...	...	...	...	76	224·9±3·42	29·7	13·2	2	
...	...	...	...	64	239·8±3·26	25·9	10·8	5	
...	...	...	...	72	242·7±2·91	24·6	10·1	7	
...	1	...	1	71	242·6±3·50	29·2	12·0	8	
...	1	...	...	72	236·4±3·08	26·0	11·0	13	
...	...	...	...	66	242·0±3·21	26·0	10·7	15	
2	1	1	...	72	251·8±3·69	31·3	12·4	18	
1	...	...	...	73	245·9±3·18	27·1	10·9	19	
...	...	...	...	59	239·9±3·53	27·0	11·2	20	
...	...	...	...	62	241·8±2·67	21·1	8·7	21	
...	...	...	...	60	243·3±3·17	24·3	10·0	22	
1	...	...	...	69	238·2±3·45	28·5	11·9	24	
...	...	...	...	60	236·8±2·96	22·8	9·6	25	
...	...	...	...	69	234·7±2·57	21·2	9·0	26	
...	...	...	...	67	242·3±3·44	27·9	11·1	28	
1	...	...	...	78	243·2±2·76	24·2	9·9	29	
...	...	...	...	69	252·5±2·81	23·2	9·2	30	
...	...	...	...	71	237·5±3·42	28·7	12·1	32	
...	...	...	...	66	248·9±3·30	26·7	10·7	33	
...	...	...	...	69	246·1±2·82	23·4	9·5	34	
...	...	...	...	69	236·9±2·90	23·9	10·0	36	
...	...	...	...	61	228·3±3·42	26·6	11·6	37	
...	...	...	...	47	229·2±2·69	18·4	8·0	38	Pure short.
...	...	...	...	70	231·4±2·46	20·4	8·8	40	
...	...	...	...	69	250·8±2·27	18·7	7·4	44	
...	...	...	...	68	257·6±2·16	17·7	6·8	50	
...	...	...	...	62	256·6±2·48	19·4	7·6	52	
...	...	...	...	68	255·4±2·39	19·7	7·7	55	
...	...	...	...	72	255·0±2·51	21·1	8·3	58	
...	...	...	...	70	253·9±2·57	21·3	8·4	61	
...	...	...	...	55	243·3±2·15	15·7	6·4	62	
4	...	...	...	68	288·8±1·74	14·2	4·9	62	

TABLE XVIII

Frequency distribution of plant height in F_3 cultures segregating for the nature of inflorescence

(The distribution in the open and crowded classes are given separately. The frequencies of the plants havin crowded pod habit are printed in *italics*.)

F_3 culture No.	Height of the parent plant in F_2 (cm.)	Frequencies under different classes of plant height in cm.																	Total population	Remarks
		175	185	195	205	215	225	235	245	255	265	275	285	295	305	315	325	335	345	
51	341 Tall	*1*	*4*	*12*	*7*	*3*	*5*	*6*	*3*	*4*	*2*	*3*	..	..	..	..	..	..	..	*50*	
		..	1	2	2	6	3	2	1	5	..	1	..	2	1	..	..	..	..	26	
141	357 „	*1*	..	*1*	*4*	*4*	*15*	*12*	*4*	*5*	*2*	*2*	..	*3*	*1*	..	..	..	..	*54*	
		..	..	..	..	..	..	2	2	2	2	1	..	..	1	..	..	..	..	10	
148	332 Short	..	..	*2*	*3*	*7*	*14*	*7*	*5*	*7*	*8*	*2*	*2*	..	..	..	..	..	..	*57*	
		..	..	..	..	..	1	2	3	1	3	1	2	1	1	..	..	..	..	15	
185	332 „	..	..	*2*	*6*	*6*	*12*	*9*	*8*	*1*	*4*	*2*	*2*	..	..	..	..	..	..	*52*	
		..	..	..	..	1	..	3	2	1	4	2	2	2	..	..	1	..	1	19	
263	318 „	..	..	*5*	*7*	*6*	*11*	*11*	*10*	*6*	*5*	..	..	..	..	..	..	..	..	*61*	
		..	..	..	..	..	1	2	1	1	1	1	..	2	1	..	1	..	..	11	

| | | |

F_2 culture No.	Height of the parent plant in F_2 (cm.)	Frequencies under different classes of plant height in cm.																	Total population	Remarks.
		175	185	195	205	215	225	235	245	255	265	275	285	295	305	315	325	335	345	
410	324 Short	..	..	4	2	6	11	13	10	3	..	..	..	..	..	..	..	..	..	49	
		..	..	..	..	..	..	1	1	3	1	2	3	4	3	..	..	..	..	18	
412	337 „	..	..	..	3	7	17	11	20	4	2	..	..	..	..	..	..	..	..	64	
		..	..	..	..	..	..	..	..	1	..	6	2	2	2	1	..	..	..	14	
413	344 Tall	..	..	1	1	2	8	3	10	15	6	..	..	..	..	..	..	..	..	46	
		..	..	..	..	..	..	3	3	2	3	4	1	4	3	..	..	..	..	23	
436	323 Short	2	1	4	6	7	7	9	12	4	5	..	..	..	..	..	..	..	..	57	
		..	..	..	..	..	..	1	1	..	4	2	2	4	..	..	..	..	..	14	
437	322 „	..	..	2	3	3	7	9	16	3	..	..	..	..	..	..	..	..	..	43	
		..	..	..	..	..	..	..	1	3	3	2	9	4	1	..	..	..	..	23	
473	342 Tall	..	..	1	1	6	8	6	11	7	1	1	1	..	..	..	..	..	..	43	
		..	..	1	..	..	1	..	6	7	2	3	3	2	1	..	..	..	..	26	
476	333 Short	2	..	1	6	7	7	12	10	7	..	..	..	..	..	..	..	..	..	52	
		..	..	..	1	..	..	1	2	4	1	7	..	1	..	..	..	..	..	17	
478	308 „	..	3	4	4	18	12	..	5	5	1	..	..	..	..	..	..	..	..	52	
		..	..	..	..	..	..	1	2	1	..	1	1	2	1	..	..	..	..	9	
485	327 „	..	..	4	4	4	11	8	3	3	2	..	..	..	..	..	..	..	..	39	Pure short.
		..	..	..	..	..	2	1	3	2	..	..	..	..	..	..	..	..	..	8	

574	318	„	.	*2*	..	*2*	*4*	*8*	*11*	*14*	*10*	..	*1*	..	..	..	..	..	..	..	*52*
				..	..	..	..	1	2	7	1	2	1	3	1	..	..	..	..	..	18
669	336	„	.	..	..	..	*2*	*1*	*3*	*11*	*19*	*14*	*7*	*2*	*1*	..	..	..	..	..	*60*
				..	..	..	..	..	..	..	1	1	..	2	2	3	..	..	..	..	9
724	327	„	.	..	..	..	..	..	..	*10*	*17*	*7*	*9*	*5*	*2*	..	..	..	..	..	*50*
				..	..	..	..	..	..	..	3	3	3	2	3	3	1	..	..	..	18
908	312	„	.	..	..	..	*2*	*1*	*3*	*6*	*7*	*8*	*11*	*13*	*2*	..	..	..	..	..	*53*
				..	..	..	..	..	..	..	2	2	1	2	2	..	..	..	..	..	9
918	322	„	.	..	..	..	..	*4*	*1*	*11*	*7*	*10*	*10*	*5*	..	..	..	..	..	..	*48*
				..	..	..	..	..	..	1	..	7	1	5	2	4	..	..	..	..	20
964	327	„	.	..	..	*2*	..	*3*	..	*10*	*11*	*9*	*11*	*3*	*3*	*1*	..	..	..	..	*53*
				..	..	..	..	..	..	1	3	1	4	6	2	2	..	..	..	..	19
1006	322	„	.	..	..	*2*	*2*	..	*5*	*2*	*8*	*16*	*9*	*4*	*3*	..	..	..	..	..	*51*
				..	..	..	..	..	2	1	1	4	4	2	4	1	..	..	..	..	19

5. Seed colour

The frequencies in F_2 indicated that brown markings such as occur in Type 80 were dominant to the Type 5 phenotype and that inheritance was on a monohybrid basis. The behaviour of cultures in F_3 is shown below :—

TABLE XIX

F behaviour of the inheritance of seed colour

No. of F_3 cultures and nature of parents in F_2	Segregation	Frequencies	
		Observed	Expected
46 cultures from plants belonging to F_1 and Type 80 phenotypes.	Pure	16	15·3
	Segregating 3 brown : one Type 5.	30	30·7
14 from plants with Type 5 seed.	Pure	14	14

The theory based on F_2 is confirmed.

In segregating cultures in F_3 we obtain a total ratio as shown below :—

——	Seed like F_1 and Type 80	Seed Type 5	Total
Observed	1514	494	2008
Expected (3 : 1)	1506	502	
Deviation	8·0		

S. E. $= 19 \cdot 40$.

$\frac{\text{Dev.}}{\text{S. E.}} = \frac{8 \cdot 0}{19 \cdot 40} = \mathbf{0 \cdot 41}$. The fit is good.

It has already been stated in the consideration of F_2 that it is very probable that the inheritance of seed colour is on a more complicated basis than a monohybrid ratio. The reason for this is that the attempt to separate the F_1 and Type 80 phenotypes in F_2 gave the following segregations in F_3.

TABLE XX

F_3 behaviour of the inheritance of seed colour

No. of F_3 cultures and nature of their parents in F_2	Segregation	Frequencies	
		Observed	Expected on dihybrid basis
36 F_1 . .	Pure F_1	2	4
	3 F_1 : 1 Type 80	4	8
	3 F_1 : 1 Type 5	10	8
	9 F_1 : 3 Type 80 : 4 Type 5 . .	20	16
10 Type 80 .	Pure Type 80	2	3·3
	3 F_1 : 1 Type 80	8	*Nil*
	3 Type 80 : 1 Type 5 . . .	*Nil*	6·7
14 Type 5 .	Pure Type 5	14	14

The segregations of cultures in F_3 from the F_1 phenotype are suggestive but the behaviour of cultures from the Type 80 phenotype shows clearly that we are unable to distinguish with certainty between F_1 seed and Type 80 seed.

The behaviour of cultures in F_3 as regards seed colour is also shown in Table XII. It will be seen that every culture which was pure for "coloured" flower was pure for brown markings on seed and that every culture which was pure for "non-coloured" flower was also pure for Type 5 phenotype seed. The evidence of the "cross-overs" also supports the idea that linkage is absolute, for in every case of an F_3 culture from a cross-over parent in F_2 it was found that a segregation was taking place which should not have existed. Thus a plant diagnosed in F_2 as having Type 5 flower and coloured seed gave in F a segregation for flower colour into three F_1 : one Type 5 phenotypes and seed colour was also segregating. As already explained the development of red in the flower is very much affected by the season, amount of sunlight, etc., and the development of colour in the seed varies somewhat with the degree of ripeness, dryness,

etc. The very few supposed cross-overs we, therefore, attribute to mistaken diagnosis in F_2 and consider that a single factor is responsible for red colour in the flower and brown markings on the seed.

Part II

The inheritance of wilt-resistance

The mode of inheritance of flower colour, inflorescence, habit, stature and seed colour has already been described and shown to follow normal Mendelian ratios. If an F_2 population is grown in an infected field then the frequency of any particular morphological phenotype will depend not only upon the Mendelian probability of occurrence of this phenotype but also upon its chances of surviving the attack of the disease. If resistance to disease is a Mendelian character and if the factor or factors for resistance are linked with any factors for morphological characters then the phenotypic frequencies in an F_2 population in an infected field will be affected by differential rates of mortality. If, however, no such linkage exists then the phenotypic frequencies in the F_2 in an infected field will not be disturbed by disease and it is possible that the proportion of survivors may indicate the nature of the Mendelian ratio involved in the inheritance of disease-resistance.

F_1 generation

F_1 was grown in artificially infected fields in 1928-29 and in 1932-33. The following table shows the percentage loss due to wilt in F_1 and in the parents in these fields.

Table XXI

Percentage loss due to wilt in F_1 and parents

Culture	Percentage loss		
	1928-29	1932-33	
	Barah plot 7	Barah plot 7	Lawn plot 2
Type 80 parent	7·3	18·6	2·2
Type 5 parent	98·7	97·3	95·5
F_1	8·3	26·5	27·9

Barah 7 had been infected for two seasons previous to 1928-29. It was used also in 1929-30 but was not infected again until 1932-33. Lawn plot 2 was infected for the first time in 1932-33. The figures show that the resistance of F_1 is much greater than that of the susceptible parent but less than that of the resistant parent. In 1928-29 the number of F_1 plants was only twelve and specially good seeds were selected for growing this population. It is possible, therefore, that a higher degree of resistance was shown by this population than is really characteristic of F_1. In 1932-33, however, the F_1 in Barah 7 consisted of 177 plants and that in Lawn plot 2 of ninety-three plants, populations large enough to give significant figures.

F_2 GENERATION

The F_2 generation was grown in 1929-30, 1932-33 and 1933-34. In the first case the F_2 was from F_1 plants which had survived in an infected field (Barah plot 7 in 1928-29); in the other two cases the F_1 plants from which the F_2 populations were raised had not been grown in infected fields.

TABLE XXII

Percentage loss due to wilt in F_2 and parents

Culture	Percentage loss			
	1929-30	1932-33		1933-34
	Barah plot 8	Barah plot 7	Lawn plot 2	Lawn plot 3
Type 80 parent . . .	47·5	18·6	2·2	14·0
Type 5 parent . . .	99·0	97·3	95·5	91·3
F_2 (Type 80 × Type 5) .	80·5	32·7	40·1	..
F_2 (Type 5 × Type 80) .	..	41·6	43·6	50·0

The loss in F_2 in Barah 8 was about eighty per cent but this field was very heavily infected having been in the experiment and under *rahar* for four successive seasons and as has been explained in a previous paper [McRae and Shaw, 1933], a resistant type even will lose its resistance under these conditions; in fact, we note that Type 80, the resistant parent, under these circumstances had a loss of forty seven per cent. In 1932-33 the F_2 was grown in fields which had not been infected with wilt for so long and, as a matter of fact, in Lawn plot 2 the infection had

been given only in that season, and the plot had never b fore been under *rahar*. The loss due to wilt in F_2 in Lawn plot 2 was from 40 to 44 per cent, in Barah 7 from 33 to 42 per cent and in Lawn plot 3 about 50 per cent. The actual figures are shown in Table XXIII given below :—

TABLE XXIII

Loss due to wilt in six F_2 populations of rahar *cross Type 80 × Type 5 and reciprocal*

Culture	1929-30		1932-33		1933-34			
	Barah plot 8		Barah plot 7		Lawn plot 3		Lawn plot 2	
	No. of wilted plants	No. of survivors	No. of wilted plants	No. of survivors	No. of wilted plants	No. of survivors	No. of wilted plants	No. of survivors
F_2 (Type 80 × Type 5).	385	93	93	191	106	158	...	...
F_2 (Type 5 × Type 80).	...	...	72	101	92	119	516	508

In fields which have been artificially infected and which have not been continuously under *rahar* for successive seasons, the loss due to wilt in F_2 ranges from 32 to 50 per cent. A loss of 43 to 44 per cent was approximated to in three cases and gives the ratio of survivors to deaths of 9 : 7 or 27 : 37 ; these proportions suggest that two or three pairs of factors are concerned in the inheritance of resistance. But F_2 data are difficult to elucidate in this respect, since in spite of the care taken to secure uniform infection of a field, susceptible plants may escape and resistant plants may suffer from an uncontrolled and adverse physiological stimulus which renders them susceptible to infection. We may leave the consideration of this point for the moment and examine the phenotypic frequencies for flower colour and other characters in these F_2 populations which were grown in infected soil and sustained losses from wilt disease.

FLOWER COLOUR

When the F_2 is grown in a wilt-infected field the four flower colour phenotypes, which have already been described, occur almost in the normal 9 : 3 : 3 : 1 proportion. The phenotypic frequencies in the six F_2 populations studied under infected conditions are shown in Table XXIV :—

TABLE XXIV

Segregation in F_2 for flower colour in infected fields

Cross	Year	Plot	Observed frequencies				Expected frequencies on 9 : 3 : 3 : 1 ratio				Total No. of plants	χ^2	P
			F_1	T. 80	T. 5	Ringed	F_1	T. 80	T. 5	Ringed			
T. 80 × T. 5	1929-30	Barah 8	44	23	18	8	52·2	17·4	17·4	5·8	93	3·1793	Between 0·30 and 0·50
,,	1932-33	Barah 7	113	39	41	17	118·1	39·4	39·4	13·1	210	1·9351	,, 0·50 and 0·70
,,	,,	Lawn 2	79	47	27	10	91·7	30·6	30·6	10·2	163	11·0110	,, 0·02 and 0·01
T. 5 × T. 80	,,	Barah 7	53	25	25	10	63·6	21·2	21·2	7·1	113	4·0734	,, 0·20 and 0·30
,,	,,	Lawn 2	73	21	20	6	67·5	22·5	22·5	7·5	120	0·7869	,, 0·80 and 0·90
,,	1933-34	Lawn 3	270	97	83	38	274·5	91·5	91·5	30·5	488	3·0383	,, 0·30 and 0·50
		Total	632	252	214	89	667·6	222·6	222·6	74·2	1,187		

It is evidentthat the incidence of disease has not caused much distortion in the expected dihybrid ratio, except in Type 80 × Type 5 in Lawn plot 2, where an excess of Type 80 type of flowers has increased the value of the χ^2 enormously. This is possibly a case of wrong diagnosis in flower colour.

Seed colour

Here again the phenotypes which have already been described occur in the infected fields in proportions which agree closely with expectations.

Table XXV

Segregation in F_2 for seed colour in infected fields

Cross	Year	Plot	Observed frequencies		Expected frequencies on 3 : 1 ratio		Total number of plants
			Seeds with brown markings like F_1 or T. 80	Seeds like T. 5	Seeds with brown markings like F_1 or T. 80	Seeds like T. 5	
T. 80 × T. 5 . .	1929-30 .	Barah 8 .	61	23	63·0	21·0	84
,, . .	1932-33 .	Barah 7 .	131	53	138·0	46·0	184
,, . .	,, .	Lawn 2 .	139	43	136·5	45·5	182
T. 5 × T. 80 . .	,, .	Barah 7 .	70	28	73·5	24·5	98
,, . .	,, .	Lawn 2 .	61	21	61·5	20·5	82
,, . .	1933-34 .	Lawn 3 .	355	114	351·75	117·25	469
Total .	...	...	817	282	824·25	274·75	1,099

Deviation = 7·25.
S. E. = 13·91.
$\frac{\text{Dev.}}{\text{S. E.}} = \frac{7·25}{13·91} = 0·52$. The fit is good.

Inflorescence

The occurrence of the crowded and open phenotypes in F_2 populations under the incidence of disease is shown in Table XXVI which gives the phenotypic frequencies of survivors. Here again there is no indication of any selective action of the disease.

TABLE XXVI

Segregation in F_2 for type of inflorescence in infected fields

Cross	Year	Plot	Observed frequencies		Expected frequencies on 3 : 1 ratio		Total number of plants
			Crowded	Open	Crowded	Open	
T. 80 × T. 5 . .	1929-30 .	Barah 8 .	64	18	61·5	20·5	82
,, . .	1932-33 .	Barah 7 .	155	55	157·5	52·5	210
,, . .	,, .	Lawn 2 .	100	41	105·75	35·25	141
T. 5 × T. 80 . .	,, .	Barah 7 .	89	25	85·5	28·5	114
,, . .	,, .	Lawn 2 .	81	41	91·5	30·5	122
,, . .	1933-34 .	Lawn 3 .	380	140	390·0	130·0	520
Total .	...	...	869	320	891·75	297·25	1,189

Deviation = 22·75.
S. E. = 34·48.
$\frac{\text{Dev.}}{\text{S.E.}} = \frac{22·75}{34.48} = 0·66$. The fit is good.

HABIT

The occurrence of the erect and spreading phenotypes among the survivors in F_2 under infected conditions also agrees with expectations as is shown in Table XXVII.

TABLE XXVII

Segregation in F_2 for plant habit in infected fields

Cross	Year	Plot	Observed frequencies		Expected frequencies on 3 : 1 ratio		Total number of plants
			Erects and Inters.	Spreaders	Erects and Inters.	Spreaders	
T. 80 × T. 5 . .	1929-30 .	Barah 8 .	71	12	62·25	20·75	83
,, . .	1932-33 .	Barah 7 .	151	59	157·50	52·50	210
,, . .	,, .	Lawn 2 .	111	37	111·00	37·00	148
T. 5 × T. 80 . .	,, .	Barah 7 .	91	23	85·50	28·50	114
,, . .	,, .	Lawn 2 .	82	39	90·75	30·25	121
,, . .	1933-34 .	Lawn 3 .	402	118	390·00	130·00	520
Total .	...	...	908	288	897	299	1,196

Deviation = 11.
S. E. = 14·54.
$\frac{\text{Dev.}}{\text{S.E.}} = \frac{11}{14·54} = 0·76$. The fit is good.

It is evident from the facts presented above that in the inheritance of flower colour, seed colour, type of inflorescence and habit, the normal phenotypic proportions among the survivors of an F_2 population under wilt disease were undisturbed and we may safely infer that the factors which are concerned with the inheritance of these characters are inherited independently of those which are concerned with the inheritance of wilt-resistance.

F_3 GENERATION

Since the phenotypic ratios for the characters studied in F_2 were not disturbed by the incidence of wilt disease in the F_2 population, we infer that the inheritance of wilt-resistance is not linked to that of these characters and that the genotypes which occur in F_2 under normal conditions also occur in the same proportions when the population is subjected to disease. With the object of further investigating this, two F_2 populations, which were grown in 1932-33, were carried to F_3 in an infected field in 1933-34. One of these F_2 populations was the complete progeny of a single F_1 plant, and was grown under normal uninfected conditions, it contained 193 individuals and will be referred to as the 'B' series. The other F_2 population was also the progeny of a single F_1 plant and was grown under infected conditions. In this population there were ninety-eight survivors, the seed of which was carried to F_3, this is referred to as the 'A' series.

Thus in the "B" series we should expect to obtain all possible genotypes in F_2 and in the "A" series we shall obtain in F_2 the genotypes which survive the incidence of wilt.

Table XXVIII shows the distribution of the loss due to wilt disease among the F_3 families of these populations and among the parent types (Types 5 and 80) in the same fields. Types 5 and 80 behave with reference to disease resistance in accordance with what has been previously described, the two F_3 populations, however, each contain cultures which cover the whole range from susceptibility to complete resistance.

TABLE XXVIII

Frequency distribution of number of cultures for different degrees of wilt infection in rahar *cross Type 5 × Type 80—F_3 in an infected field*

Year and series	Plot	Seed	Number of cultures with different percentages of wilted plants — Up to per cent											Total	Remarks
			0	10	20	30	40	50	60	70	80	90	100		
1933-34 A Series	Lawn 2 and part of Lawn 1	Type 5	...	...	...	...	...	...	...	...	...	...	9	9*	* Distribution of 9 rows of this type in A series and of 24 rows in B series.
		Type 80	7	3	1	1	...	...	...	...	...	...	...	12†	† Distribution of 12 rows of this type of A series and of 2 rows in B series.
		T. 5 × T. 80-F_3	2	2	8	13	9	9	18	15	14	7	1	98‡	‡ Distribution of different F_3 families, each family in one row.
1933-34 B Series.	Lawn 1	Type 5	...	...	...	...	...	1	2	2	1	1	17	24*	
		Type 80	2	...	...	...	...	...	...	...	...	...	...	2†	
		T. 5 × T. 80-F_3	1	11	20	23	18	30	16	18	10	10	36	193‡	

Each culture occupied one row in the field and contained thirty to forty plants. In Series A, therefore, there were nine rows of Type 5 with 100 per cent loss and eight F_3 cultures with 20 per cent loss.

In ninety-eight cultures in F_3 from the survivors of the F_3 which were grown under infection (the "A" series), there were two families which had no deaths due to wilt, and which may be classed as very resistant, fifteen families which sustained a loss of 70 per cent due to wilt and one family which was completely destroyed by this disease. In the 193 cultures in F_3 from the complete F_2 which was grown under non-infected conditions (the "B" series), one family had no deaths, eighteen families had a loss of 70 per cent and thirty-six families were almost completely destroyed by wilt. These frequencies suggest that in the A-series most of the susceptible plants had been destroyed by disease in F_2; these susceptible plants were probably individuals which were either heterozygous for factors for resistance or homozygous for their complete absence, in fact they were the non-resistant genotypes. In the A-series about one per cent of the total number of families have a death rate of over ninety per cent but in the B-series, in which the elimination of the more susceptible genotypes in F_2 had not taken place, about eighteen per cent of the total number of cultures have a death rate of over ninety per cent. It follows that in the F_2 grown under infected conditions (A-series), the completely susceptible genotypes were destroyed and the resulting F_3 population is from almost entirely resistant and partially resistant genotypes. In both series the distribution of resistance and susceptibility among the F_3 families shows that the inheritance of wilt-resistance is dependent upon multiple factors.

It has already been shown from the inheritance of morphological characters in F_2 and F_3 that there is no linkage between the heritable factors for wilt-resistance and those for the morphological characters studied. From our knowledge of the genotypes which are phenotypically represented in F_3, it is possible to see how the loss due to wilt disease is distributed among the different genotypes. Thus for instance, if, in F_3, cultures which were "pure open" for the inflorescence character, appear uniformly to maintain a very low loss due to wilt, we should infer that this morphological phenotype was correlated or linked with disease resistance. If, however, in families which are pure for this morphological phenotype, and in other families in F_3 pure for some other character (*e.g.*, "pure F_1 flower"), all grades of resistance occur, we obtain further proof of the absence of linkage and the dependence of resistance upon multiple factors. This, as a matter of fact, is the case and is shown clearly in Tables XXIX to XXXII.

TABLE XXIX

The distribution of loss due to wilt among F_3 families in relation to the inheritance of the type of inflorescence

Phenotypes	F_3 Series	No. of cultures and percentages of wilted plants in each culture — Up to per cent											No. of families	Remarks
		0	10	20	30	40	50	60	70	80	90	100		
Pure open	A	...	...	2	3	4	2	6	2	2	1	...	22	Families with very small populations in Series B have not been considered in this comparison.
	B	1	3	3	7	2	8	4	6	1	...	4	39	
Pure crowded	A	2	...	1	2	1	2	5	2	5	1	...	21	
	B	...	4	4	4	3	6	1	1	2	2	1	28	
Segregating 3 crowded : 1 open).	A	...	2	5	8	4	5	7	11	7	5	...	54	
	B	...	3	13	12	13	15	11	11	7	9	8	102	

It is evident from Table XXIX that all grades of resistance occur in all genotypes both in "A" and "B" series.

TABLE XXX

The distribution of loss due to wilt among F_3 families in relation to the inheritance of flower colour

Phenotypes	F_3 Series	No. of cultures and percentages of wilted plants in each culture — Up to per cent											No. of families	Remarks
		0	10	20	30	40	50	60	70	80	90	100		
Pure F_1	A	...	...	1	...	...	...	1	...	...	1	...	3	Families with very small population in Series B have not been considered in this comparison.
	B	...	1	...	4	1	...	1	1	1	...	...	9	
3 F_1 : 1 T. 80	A	...	...	...	...	...	2	1	3	3	1	...	10	
	B	...	...	6	4	...	3	1	3	1	2	2	22	
3 F_1 : 1 T. 5	A	...	...	...	3	1	...	2	1	...	...	...	7	
	B	...	1	1	1	1	...	1	1	...	3	3	12	
9 F_1 : 3 T. 80 : 3 T. 5 : 1 Ringed.	A	2	2	3	3	4	2	4	5	3	...	...	28	
	B	...	3	7	7	5	11	6	2	4	3	2	50	
T. 80 Pure	A	...	...	1	...	...	...	1	2	1	1	...	6	
	B	...	...	2	2	3	4	1	1	...	1	...	14	
3 T. 80 : 1 Ringed	A	...	...	3	3	1	2	4	1	1	1	...	16	
	B	1	3	...	1	4	3	2	1	1	1	...	17	
T. 5 Pure	A	...	...	...	2	...	...	...	...	2	2	...	6	
	B	...	1	...	...	1	...	1	2	...	...	1	6	
3 T. 5 : 1 Ringed	A	...	...	...	2	3	2	3	3	...	1	...	14	
	B	...	...	3	2	2	4	2	6	3	...	...	22	
Ringed Pure.	A	...	...	...	...	...	1	2	...	4	...	1	8	
	B	...	1	1	2	1	4	1	1	...	...	...	11	

TABLE XXXI

The distribution of loss due to wilt among the F_3 families in relation to the inheritance of seed colour

Phenotypes	F_3 families	No. of cultures and percentages of wilted plants in each culture — Up to per cent										No. of families	Remarks
		0	10	20	30	40	50	60	70	80	90	100	
Seeds with brown markings—Pure.	A	...	...	2	...	...	2	3	5	3	3	...	18	Families with very small populations in Series B have not been considered in this comparison.
	B	...	1	8	12	3	6	5	5	2	2	1	45	
Seeds with 3 brown markings: 1 silver-white segregating.	A	2	2	6	9	6	4	10	7	5	1	...	52	
	B	1	7	8	7	11	14	7	4	6	4	1	70	
Seeds silver-white—Pure.	A	...	...	...	4	3	3	5	3	6	3	1	28	
	B	...	2	4	4	4	8	5	7	2	...	3	39	

TABLE XXXII

The distribution of loss due to wilt among F_3 families in relation to the inheritance of plant habit

Phenotypes	F_3 families	No. of cultures and percentages of wilted plants in each culture — Up to per cent										No. of families	Remarks.
		0	10	20	30	40	50	60	70	80	90	100	
Erect—Pure .	A	...	...	1	3	1	...	2	2	3	...	1	13	Families with very small populations in Series B have not been considerred in this comparison.
	B	...	1	1	3	1	4	1	...	2	4	2	19	
Segregating 3 Erects and Inters : 1 Spreader.	A	2	1	6	2	5	6	12	12	6	4	...	56	
	B	1	7	13	13	13	21	11	11	7	7	7	111	
Spreader—Pure .	A	...	1	1	8	3	3	4	1	5	3	...	29	
	B	...	1	6	6	4	4	4	7	1	...	1	34	

Considering Tables XXIX to XXXII it is evident that the distribution of resistance to wilt disease among F_3 families is not dependent upon the genotypes for morphological phenotypes, which occur in these families. Taking for instance, the inflorescence character (Table XXIX) we notice that in both the A and B series all kinds of F_3 families, pure dominants, recessives and heterozygotes, show all grades of susceptibility. Considering Table XXVIII we note that in the B series a far larger total number of families in F_3 were completely destroyed by wilt, or destroyed to such an extent that no genetical deductions could be

based on the few survivors, than in the A-series; these families of the B-series appear in Table XXVIII but they cannot appear in Tables XXIX to XXXII because it was impossible, owing to the small population or to the complete elimination of plants, to assign to them a probable genetical position in these tables. The total number of cultures shown for the B-series in Table XXIX is 169 whereas in Table XXVIII the B-series contains 193 cultures. All that can be certain is that the frequencies in the columns for 70 to 100 per cent loss due to wilt in the B-series in Tables XXIX to XXXII would be increased if these twenty-four cultures could be included in these Tables (Tables XXIX—XXXII).

Further proof of the correctness of the views based on the two F_2 populations described above is furnished by the subsequent generations derived from the first F_2 population of 1929-30 (Table XXXIII). In this cross there were ninety-three survivors in F_2, of which fifty-two had vigorous seed production and were selected as parents of F_3 cultures. The loss due to wilt disease was distributed among these fifty-two F_3 cultures as follows :—

TABLE XXXIII

Number of cultures from F_2, 1929-30 with different percentages of wilted plants in Barah plot 6

Culture	Percentage deaths						
	Up to per cent						
	0	10	20	30	40	—	80
F_3 (1930-31)	20	11	15	5	1		

Type 5 parent . . . . . 74·3 per cent loss.
Type 80 parent . . . . . 0·9 per cent loss.

It will be apparent that in this F_3, twenty families were completely resistant and that no family had a loss exceeding forty per cent. This is very interesting as it will be remembered that the F_2 of this cross was grown in a very heavily infected field and sustained a loss of about eighty per cent; that is to say, in F_2 practically all susceptible and partially susceptible genotypes were eliminated—hence this cross shows a much higher degree of resistance in F_3 than those previously described.

The distribution of loss due to wilt among these fifty-two F_3 families in relation to morphological characters is shown in Table XXXIV from which it will be apparent, as in the previous cases, that the distribution of loss was not correlated with any morphological character.

TABLE XXXIV

Distribution of loss due to wilt among F_3 families (1930-31) in relation to the inheritance of other characters

(Nature of segregations in F_3 families)

Percentage of wilted plants	Plant habit			Inflorescence			Flower colour							Seed colour					
	Erect	Spreader	Segregating Erect and Inters: Spreader	Crowded	Open	Crowded: Open	F_3	T. 80	T. 5	Ringed	F_1: T. 80	F_1: T. 5	F_1: T. 80: T. 5: Ringed	T. 80: Ringed	T. 5: Ringed	Brown markings	Silver-white	Brown silver-white	No. of F_3 families in each grade of resistance
0 . .	1	8	11	5	3	12	2	1	2	2	4	2	1	2	4	8	8	4	20
Upto 5 per cent	...	1	4	1	2	2	1	...	...	...	...	...	2	1	1	1	1	3	5
„ 10 „ .	1	1	4	...	1	5		1	...	1	...	2	1	1	...	1	1	4	6
„ 20 „ .	1	...	14	2	3	10	...	3	...	1	3	2	3	2	1	7	2	6	15
„ 30 „ .	...	1	4	1	...	4	...	...	...	...	...	2	2	...	1	...	1	4	5
„ 40 „ .	...	...	1	...	...	1	...	...	1	...	...	...	...	...	...	...	1	...	1
Total .	3	11	38	9	9	34	3	5	3	4	7	8	9	6	7	17	14	21	52

Selected families from this particular cross have been carried up to the F_6 generation, some details of which are given below.

Four F_3 families were selected from this cross of 1929-30 for further study and the F_4 generation was grown in the same plot as the F_3, *viz.*, Barah 6 in 1931-32.

F_4 GENERATION

Selection in F_3 was made so as to secure various combinations of different morpholcgical characters with wilt-resistance. Special stress was, however, given to selecting plants with erect habit, because *rahar* varieties with such habit are preferred on account of the ease in inter-cultivation, harvesting and threshing. Families B-69, D-22, E-4 and F-60 were selected for the purpose and their F_2 and F_3 characters are tabulated below :—

TABLE XXXV

F_2 and F_3 behaviour of selected families

Family	Percentage of wilted plants in F_3	Plant habit		Inflorescence		Flower colour		Seed colour	
		F_2	F_3	F_2	F_3	F_2	F_3	F_2	F_3
B-69	0	Spreader	Spreader	Open	Open	Ringed	Ringed	Silver-white like T. 5	Silver-white like T. 5
D-22	8	Erect	Erect	Crowded	Crowded : Open	T. 80	T. 80	Brown like F_1	Brown like F_1
E-4	16	Erect	Erect	Crowded	Crowded : open	T. 5	T. 5 : Ringed	Silver-white like T. 5	Silver-white like T. 5
F-60	0	Erect	Erect	Open	Open	F_1	F_1 : T. 80	Brown like T. 80	Brown like T. 80

It will be seen from the above table that family B-69 had no deaths due to wilt and was genetically pure for all the morphological characters studied. The remaining three families, on the other hand, each segregated for some particular morphological character or the other. Thus D-22 segregated for inflorescence while E-4 segregated for inflorescence and flower colour. A number of plants were selected from each of these segregating families as well as from B-69, which was pure for all characters, and these were carried on to F_4 generation.

E-4 was pure in F_3 for erect habit and silver-white seed colour like Type 5, but segregated for type of inflorescence and flower colour. The following table shows the characters of the palnts as regards inflorescence and flower colour selected in this family as parents for F_4.

TABLE XXXVI

Characters of plants selected in F_3

Nature of inflorescence	Flower colour		Total number of plants
	Type 5	Ringed	
Crowded	3, 5, 6, 18, 25	11, 14, 16	8
Open	4, 8, 13	19	4
Total .	8	4	12

Figures in columns 2 and 3 in Table XXXVI denote plant numbers in F_3 families.

It will be apparent that five plants with crowded inflorescence and Type 5 flowers were selected, three with crowded inflorescence and ringed flowers, three with open inflorescence and Type 5 flowers and one plant with open inflorescence and ringed flowers.

F-60 was pure for erect habit, open type of inflorescence and brown seeds like Type 80, but segregated for flower colour. In this family twelve plants with F_1 flower colour and twelve plants with Type 80 flower were selected. The numbers of these plants being :—

F_1 flower colour . . Plant Nos. 3, 4, 7, 8, 10, 11, 12, 13, 14, 16, 18 and 24.

Type 80 flower colour . Plant Nos. 1, 2, 5, 9, 15, 17, 19, 20, 21, 22, 23 and 25.

D-22 was pure for erect habit, flower colour like Type 80 and brown seeds like F_1 but segregated for crowded and open types of inflorescence. Ten plants were selected in this family as follows :—

Crowded inflorescence . Plant Nos. 3, 6, 11, 17, 18 and 23.

Open inflorescence . Plant Nos. 5, 15, 16 and 19.

B-69 was pure for all morphological characters studied and hence only seven plants were selected from this family.

That multiple factors are responsible for the inheritance of wilt-resistance is further shown by the distribution of these fifty-three cultures in F_4 with reference to the loss due to wilt :—

TABLE XXXVII

Distribution of F_4 families according to deaths due to wilt

Family	Percentage of wilted plants in F_3 cultures	Frequency of families with different percentages of wilt in F_4								Total number of families
		Upto per cent								
		0	10	20	30	40	50	—	100	
F-60 . . .	0	9	10	4	1	..	..	—	..	24
D-22 . . .	8	5	..	1	1	1	1	—	1	10
E-4 . . .	16	1	1	5	3	1	1	—	..	12
B-69 . . .	0	5	2	..	..	..	..	—	..	7

Type 80 parent . . . . . 0 per cent
Type 5 parent . . . . . 79·8 per cent

It will be seen that families F-60 and B-69 which showed no deaths due to wilt in the F_3 had many cultures in F_4 with either no deaths or with not more than ten per cent of deaths. Nine families in F-60 showed no deaths while fifteen families had deaths ranging from ten to thirty per cent. In the case of families D-22 and E-4 which had eight per cent and sixteen per cent deaths in F_3 respectively, the range of distribution was much wider as is shown in the above table.

The distribution of F_4 families for the different morphological characters studied in relation to the percentage of wilted plants in each family is tabulated below :—

TABLE XXXVIII

Distribution of loss due to wilt among F_4 families (1931-32) in relation to the inheritance of other characters

Family	Percentage of wilted plants	Plant habit			Inflorescence			Flower colour								Seed colour			No. of families in each series
		Erect	Spreader	Erect : Spreader	Crowded	Open	Crowded : Open	F_1	T. 80	T. 5	Ringed	F_1 : T. 80	F_1 : T. 5	F_1 : T. 80 : T. 5 Ringed	T. 80 : Ringed	T. 5 : Ringed	Brown marking	Silver-white	Brown : silver-white	
F-60	0	9	...	...	...	9	...	2	4	...	...	2	1	...	...	...	9	...	...	
	5	6	...	...	...	6	...	...	3	...	...	3	...	...	...	...	6	...	...	6
	10	4	...	...	...	4	...	1	1	...	...	2	...	...	...	...	4	...	...	4
	20	4	...	...	...	4	...	...	2	...	1	1	...	...	...	...	4	...	...	4
	30	1	...	...	...	1	...	...	1	...	...	...	...	...	...	...	1	...	...	1
E-4	0	1	...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	1	...	1
	5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	10	1	...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	1	...	1
	20	5	...	...	2	2	1	...	...	2	3	...	...	...	...	...	...	5	...	5
	30	3	...	...	1	...	2	...	...	2	...	...	...	...	...	1	...	3	...	3
	40	1	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	1	...	1
	50	1	...	...	...	1	...	...	...	...	...	...	...	...	...	1	...	1	...	1
D-22	0	5	...	...	3	2	...	...	5	...	...	...	...	...	...	...	5	...	...	5
	5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1
	20	1	...	...	...	...	...	...	1	...	...	...	...	...	...	...	1	1	...	1
	30	1	...	...	...	1	...	...	1	...	...	...	...	...	...	...	1	...	...	1
	40	1	...	...	...	1	...	...	1	...	...	...	...	...	...	...	1	...	...	1
	50	1	...	...	...	...	1	...	1	...	...	...	...	...	...	...	1	...	...	1
	100	1	...	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...
B-69	0	...	5	...	...	5	...	...	...	...	5	...	...	...	...	...	...	5	...	5
	5	...	2	...	...	2	...	...	...	...	2	...	...	...	...	...	...	2	...	2

F_5 GENERATION

A notable feature in the study of the F_5 generation was that not only the promising wilt-resistant hybrids descended from the families B-69, D-22, E-4, and F-60 were carried to F_5 but that five other families (A', B', C', D', E') of the same cross which were similar in morphological characters to the above but in which the previous generations had never been grown in fields artificially infected with wilt (the B-series— see page 172), were also included in order to determine their response to wilt.

The following cultures were selected for growing in F_5 :—

TABLE XXXIX

Description of selected cultures for F_5

Family	Cultures	Characters			Seed colour	Morphologically similar parents selected from F_4 in the B-series
		Plant habit	Inflorescence	Flower colour		
B-69 . .	A.5	Spreading	Open .	Ringed .	T.5 .	A'.240
E-4 . .	B.14	Erect .	Crowded	Ringed .	T.5 .	B'.620
E-4 . .	C.15	Erect .	Open .	T.5 .	T.5 .	C'.1055
D-22 . .	D-24 .	Erect .	Crowded	T.80 .	F_1 Brown	D'.514
D-22 . .	D-30 .	Erect .	Crowded	T.80 .	F_1 Brown	..
D-22 . .	D-32 .	Erect .	Crowded	T.80 .	F_1 Brown	..
F-60 . .	E-50 .	Erect .	Open .	T.80 .	T.80 Brown.	E'.562
F-60 . .	E-39 .	Erect .	Open .	T.80 .	T.80 Brown.	—
F-60 . .	E-57 .	Erect .	Open .	T.80 .	T.80 Brown.	—

The F_5 series A', B', C' D', E' had, therefore, been bred without any selection for resistance to wilt and were respectively morphologically similar to the F_5 cultures A-5, B-14, C-15, D-24, 30, 32, E-39, 57, 59 which had been bred for wilt-resistance. Table XXXIX makes clear the position of these F_5 cultures.

Cultures 28 and 38 derived from wilt-resistant families, D-51 and C-14 (from A series) respectively were also grown. Their morphological characters are shown below :—

TABLE XL

Description of two other wilt-resistant cultures

Family	Cultures	Plant habit	Characters		
			Inflorescence	Flower colour	Seed colour
D-51 . .	28	Erect .	Open .	T.80 .	Brown like F_1
C-14 . .	38	Erect .	Open .	F_1 . .	Brown like F_1 and A_2

About three to five cultures of each family in Tables XXXIX and XL were grown in F_5 ; the seed of a single plant being sown in one row. The average loss due to wilt, in the field in which this F_5 was grown, sustained by the original parents was 84·9 per cent for Type 5 and 0·82 per cent in the case of Type 80. This F_5 was grown in a field which had been artificially infected for the first time.

TABLE XLI

Nature of wilt-infection in F_5 families, 1932-33

Previous generations grown in a wilt-infected field					Previous generations grown in a non-infected field				
Culture	Row No.	No. of effective plants	Deaths due to wilt		Culture	Row No.	No. of effective plants	Deaths due to wilt	
			No. of plants	Percentage				No. of plants	Percentage
A.5 .	2	46	1	2·17	A'.240 .	3	45	2	4·44
	35	44	3	6·82		36	46	1	2·17
	69	47	3	6·38		38	42	0	0
						71	44	1	2·27
B.14 .	4	46	3	6·52	B'.620 .	5	46	4	8·70
	33	48	3	6·25		34	47	46	97·90
	39	45	0	0		41	44	0	0
	67	45	1	2·22		68	46	33	71·80
C.15 .	6	47	16	34·05	C'.1055	32	34	32	94·20
	31	38	38	100·00		43	46	45	97·90
	37	45	42	93·40		7	45	45	100·00
	65	43	18	41·90		66	48	43	89·60
	42	45	0	0					

TABLE XLI—(*contd.*)

Previous generations grown in a wilt-infected field					Previous generations grown in a non-infected field.				
Culture	Row No.	No. of effective plants	Deaths due to wilt		Culture	Row No.	No. of effective plants	Deaths due to wilt	
			No. of plants	Percentage				No. of plants	Percentage
D.24	8	45	3	6·67	D'.514 .	9	45	44	97·80
	28	45	3	6·67		29	46	45	97·90
	44	45	0	0		45	41	38	92·79
	63	42	0	0		64	41	38	92·79
D.30	12	48	0	0					
	26	47	1	2·13					
	47	47	0	0					
	61	47	1	2·13					
D.32 .	13	46	0	0					
	25	44	1	2·27					
	48	47	1	2·13					
E.50	16	46	0	0	E'.562 .	17	46	44	95·70
	21	45	0	0		22	41	37	90·30
	52	46	0	0		53	46	43	93·50
	56	47	0	0		57	46	38	82·60
E.39 .	18	45	0	0					
	19	46	0	0					
	54	47	1	2·13					
	55	46	0	0					
28 . .	11	45	0	0	Type 80	77	43	0	0
	27	45	0	0		83	43	0	0
	46	44	0	0		88	40	0	0
	62	46	0	0		95	38	2	5·26
						101	42	0	0
38 . .	14	44	0	0		107	44	0	0
	24	43	0	0		113	47	0	0
	49	44	0	0		115	47	1	2·13
	59	43	0	0		116	48	0	0
					Total .		392	3	0·76
					Type 5 .	1	43	42	97·7
						10	46	46	100·0
						20	45	44	97·8
						30	46	46	100·0
						40	46	44	95·7
						50	46	46	100·0
						60	45	39	86·8
						70	42	35	83·4
						73	39	32	82·1
						79	34	31	91·1
						85	40	26	65·0
						91	42	37	88·2
						97	39	33	84·7
						103	40	36	90·0
						109	45	42	93·4
					Total .		638	579	90·75

The above table reveals the following facts :—

(1) That the cultures in the A (5) series showed a range of 2·17 to 6·82 per cent of deaths due to wilt while those in the A' (240) series had deaths ranging from 0 to 4·44 per cent. Both these morphologically similar phenotypic series may, therefore, be considered as wilt-resistant. The morphological characters in this group were spreading, open, ringed, T.5 seed, of which only the first two belong to the resistant parent.

(2) That the cultures in the B (14) series had 0 to 6·52 per cent deaths while those in B' (620) series had a range of 0 to 97·90 per cent. This shows that the B series was nearly pure for wilt resistance but that the B' series was segregating for this character, although both B and B' were identical for the morphological characters studied. The morphological characters in this group were erect, crowded, ringed, T. 5 seed, most of these characters belong to the susceptible parent.

(3) That the cultures in the C (15) series exhibited a range of 0 to 100·0 per cent deaths due to wilt, and the hybrids in the parallel series C'(1055) a range of 89 per cent to 100 per cent deaths, suggesting that the series C contained some cultures which were resistant, some which were very susceptible and some which were partially susceptible and that the series C' was almost fully susceptible to wilt. Of the morphological characters in this group only one character, open inflorescence, belonged to the resistant parent.

(4) That the cultures in the D series were highly resistant to wilt is shown by D (24) having 0 to 6·67 per cent deaths, D (30) showing 0 to 2·13 per cent and D (32) showing a similar 0 to 2·27 per cent deaths due to wilt. The cultures in the corresponding series D' (514), on the othar hand, all showed a very high degree of susceptibility, *viz.*, 92·79 to 97·90 per cent, suggesting that the two series were absolutely dissimilar in their power of resistance to wilt disease although they were similar to one another in other morphological characters, two of which belonged to the susceptible parent.

(5) Similarly all the cultures in series E were highly resistant to the disease but their morphologically similar phenotypes in series E' (562) were very susceptible. All the cultures in E (50) had no deaths and one death only occurred in a culture in E 39 and one in a culture in E 57. The cultures in E' (562) had deaths ranging from 82·60 to 95·70 per cent. In this group all the characters except the erect habit belonged to the resistant parent.

Cultures 28 and 38 also sustained no loss due to wilt, they were pure for the erect character of the susceptible parent.

The foregoing details again confirm our previous observations that the wilt-resistant property in this *rahar* cross segregates quite independently of all the morphological characters studied. Had there been any correlation of morphological characters with resistance or susceptibility to wilt, similar phenotypes derived either from wilt-infected or non-infected fields would have responded similarly to wilt-infection.

Plate XXVI shows graphically the percentage of deaths due to wilt shown by the different F_5 cultures as well as by different rows of the two parents.

Summary and conclusions

(1) The paper describes the inheritance of certain morphological characters and the inheritance of wilt-resistance in a cross between two unit species of *Cajanus indicus*. The types used in this cross were the Pusa Types T.5 and T.80.

(2) The inheritance of flower colour followed a 9 : 3 : 3: 1 ratio—the F_1 and the double recessive being new phenotypes unlike either parent.

(3) Erect habit was partially dominant to the spreading habit on a 3 : 1 ratio.

(4) Short stature was dominant to tall on a 3 : 1 ratio.

(5) The crowded habit of the inflorescence was dominant to the open on a 3 : 1 ratio.

(6) The brown seed of T. 80 was dominant to the silver-white of T. 5 on a 3 : 1 ratio although in this case we suspect a more complicated system of inheritance but the impossibility of diagnosing the F_1 phenotype correctly led to the adoption of the simple 3 : 1 theory.

(7) The F_2 and F_3 populations were grown in infected fields and the loss due to wilt in F_2 suggests that the inheritance of resistance may be found in a 9 : 7 or 27 : 37 ratio, resistance being dominant.

(8) The ratios of the various phenotypes in F_2 were not disturbed by the incidence of the disease showing that the inheritance of resistance was not linked with that of any of the morphological characters studied.

(9) Phenotypes which possessed the quality of resistance to wilt disease and most of the morphological characters of the susceptible parent were isolated from the cross (Table XLI, Culture B·14, Row 39).

(10) Morphologically identical hybrids showed great variation in their behaviour to wilt disease, from almost complete resistance to complete susceptibility.

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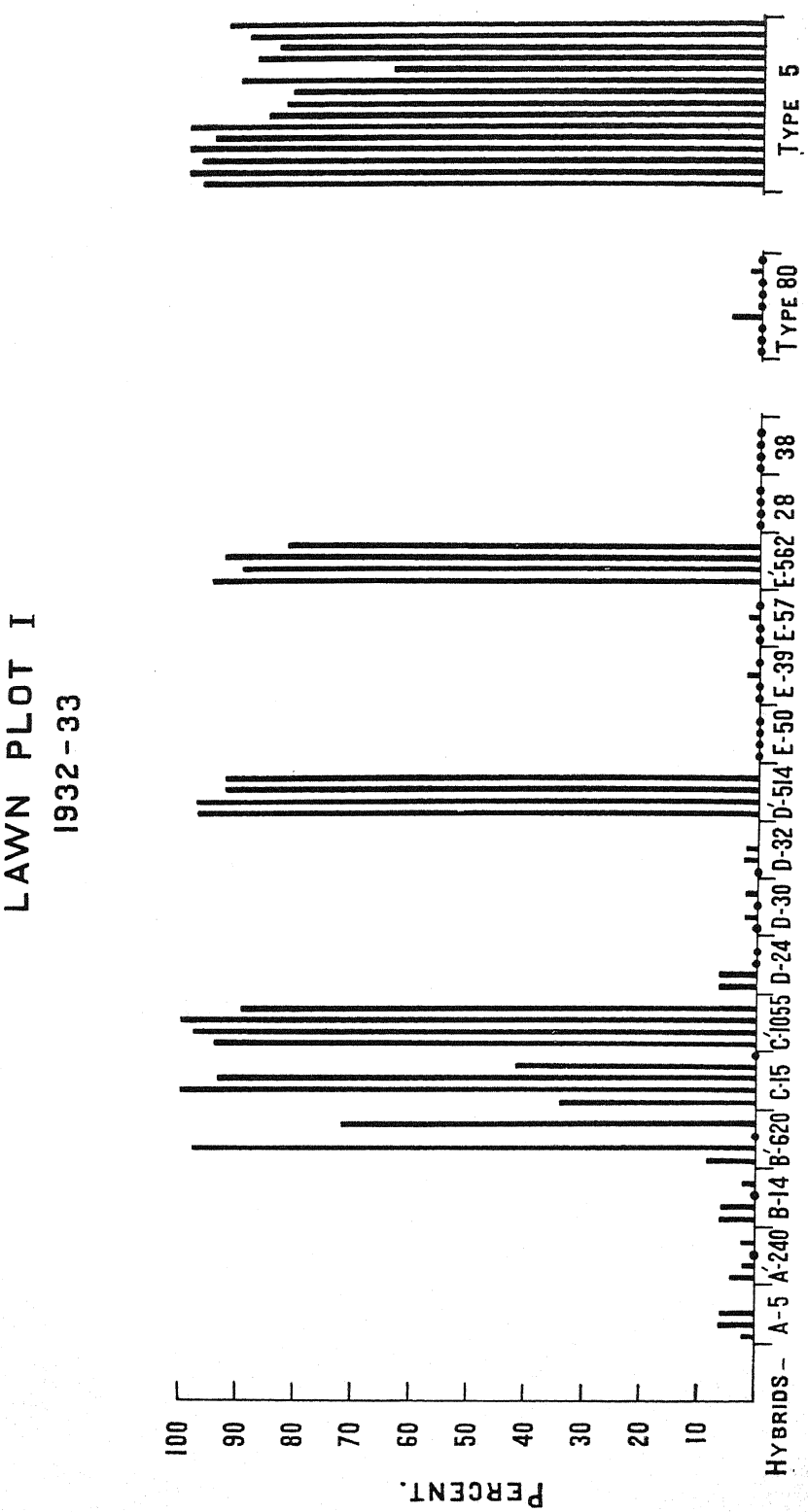

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STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.*

II. THE BIOLOGY OF THE DESERT LOCUST WITH SPECIAL RELATION TO TEMPERATURE

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CONTENTS

	PAGE
1. INTRODUCTION	189
2. PRE-COPULATION AND PRE-OVIPOSITION PERIOD	190
(*i*) Pre-oviposition period at constant temperatures	190
(*ii*) Pre-oviposition period at a temperature varying between 28° and 32°C	193
(*iii*) Pre-oviposition period of the over-wintered adults at laboratory temperature	193
(*iv*) Pre-oviposition period of the summer brood at laboratory temperature during April—July	195
3. INTER-OVIPOSITION PERIOD	196
(*i*) Inter-oviposition period of females kept at constant temperatures	196
(*ii*) Inter-oviposition period in adults kept in the hot house at a temperature varying between 28° and 32°C.	198
(*iii*) Inter-oviposition period at laboratory temperature during different months of the year	199
(*iv*) Winter intervening between two ovipositions	203

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PAGE

4. NUMBER OF OVIPOSITIONS BY A FEMALE 203
5. OVIPOSITION 204
(*i*) Oviposition at high soil temperature 204
(*ii*) Oviposition at low soil temperature 207
6. INCUBATION 209
(*i*) Incubation of eggs at constant temperatures 210
(*a*) Threshold of development 211
(*b*) Incubation period at constant temperatures . . . 214
(*c*) Vital limits for the eggs 218
(*ii*) Incubation period at alternating temperatures 219
(*iii*) Incubation period under varying temperatures in breeding cages kept in the open 221
(*iv*) Incubation period at laboratory temperature during different months 223
7. DURATION OF LARVAL STAGES 226
(*i*) Duration of larval stages at constant temperatures . . . 227
(*ii*) Upper vital limit 243
(*iii*) Duration of larval stages at laboratory temperature . . . 245
(*iv*) Duration of larval stages in the case of hoppers caged on plants in the open, during winter 247
8. LONGEVITY OF THE WINGED STAGE 249
9. TOTAL LIFE AND NUMBER OF BROODS IN A YEAR 259
10. SUMMARY 261
REFERENCES 261

1. Introduction

It is now fully recognized that without a thorough study of the biology and, more particularly, the ecology of a pest it is not possible to evolve a scientific system of prevention and control. This is particularly true of such sporadic pests as the Desert Local. During a locust visitation all energies are directed towards the destruction of this scourge and when it is over everything is forgotten. Moreover, the Desert Locust lives permanently in more or less uninhabited tracts, and out of sight is out of mind. Appreciating the importance of a thorough study of the Desert Locust from every aspect, the Imperial Council of Agricultural Research in India initiated and financed, in 1930, a scheme for locust research on an all-India basis. A part of the programme of this scheme consisted in the study of the influence of environmental factors on the bionomics of the Desert Locust. The present paper deals with one aspect of this investigation, *viz.*, the influence of temperature—one of the most important

factors in the environmental complex of the locust—on the various stages of this insect. Studies at constant temperatures have been supplemented by a large number of observations at room temperature, and even under natural environmental conditions.

The locusts employed in these experiments were obtained, both in the adult and hopper stages, from the swarms visiting the Punjab during the winter of 1930, and their progeny bred in the laboratory under different conditions. Later on collections of the 'solitary' phase adults were made from the permanent breeding grounds in Sind and South Baluchistan.

Unfortunately we started our investigations at the close of the last swarming cycle and have not been able to obtain sufficient material for as thorough an investigation as we desired. We have already greatly delayed the publication of these results in the expectation of another visitation. However, as there appears no immediate prospect of this, and, as we believe, that the results obtained would be useful to other workers, in India and elsewhere, we are publishing them.

Our thanks are due to the Imperial Council of Agricultural Research for providing funds.

2. Pre-copulation and Pre-oviposition period of *Schistocerca Gregaria*

The female locusts usually begin to oviposit almost immediately after copulation. In fact very frequently the female begins her digging operations and egg-laying while the male is still on her back. Therefore, for all intents and purposes, the pre-copulation and pre-oviposition periods (*i.e.*, the period between the last larval moult and the first coitus and first oviposition), are identical. Varying opinions have been expressed regarding the duration of this period in *Schistocerca gregaria*. Bodenheimer [1929] considers that for a normal development a full year is required, of which the pre-oviposition period is ten months. According to Regnier [1926] copulation takes place forty to fifty days after the last larval moult. Telenga [1930] states that the adults begin to pair a month after acquiring wings. Cotes [1891] and Vosselar [1905] observed the first oviposition two to three weeks after the last moult. Such widely differing statements may be accepted as correct, because evidently observations by the different investigators were made under different environmental conditions. Unfortunately no mention of these conditions has been made.

I. Pre-oviposition period at constant temperatures

The results of observations on locusts bred at constant temperatures are given in Table I and Fig. 1.

TABLE I

Pre-oviposition period at constant temperatures (1931 & 1932)

Reference No.	Temperature to which adults exposed (°C.)	Date of acquiring wings	Date of first oviposition	Pre-oviposition period (days)
(Hoppers bred crowded at 40°C.; 1932)				
40/I A2 . .	40	29th Jan. .	11th Feb. .	13
40/I A3 . .	40	29th Jan. .	11th Feb. .	13
40/II A1 . .	40	14th March .	31st March .	17
40/II A2 . .	40	14th March .	28th March .	16
			Average .	14·2
(Hoppers bred crowded at 36°C.; 1931)				
N_1 . .	36	13th March .	1st April .	19
N_2 . .	36	17th March .	31st March .	14
N_4 . .	36	17th March .	31st March .	14
N_5 . .	36	17th March .	30th March .	13
N_3 . .	36	19th March .	1st April .	13
T_2 . .	36	14th May .	2nd June .	19
T_3 . .	36	17th May .	5th June .	19
III_3 . .	36	13th July .	3rd Aug. .	21
III_2 . .	36	14th July .	4th Aug. .	21
III_1 . .	36	14th July .	6th Aug. .	23
			Average .	17·6
(Full-grown hoppers collected from a swarm; 1931)				
30 A . .	30	10th Jan. .	5th Feb. .	26
29 C . .	30	10th Jan. .	7th Feb. .	28
29 A . .	30	10th Jan. .	27th Jan. .	17
30 B . .	30	10th Jan. .	5th Feb. .	26
			Average .	24·2
(Hoppers bred crowded at room temperature; 1931)				
L 66 . .	27	18th Jan. .	19th Feb. .	32

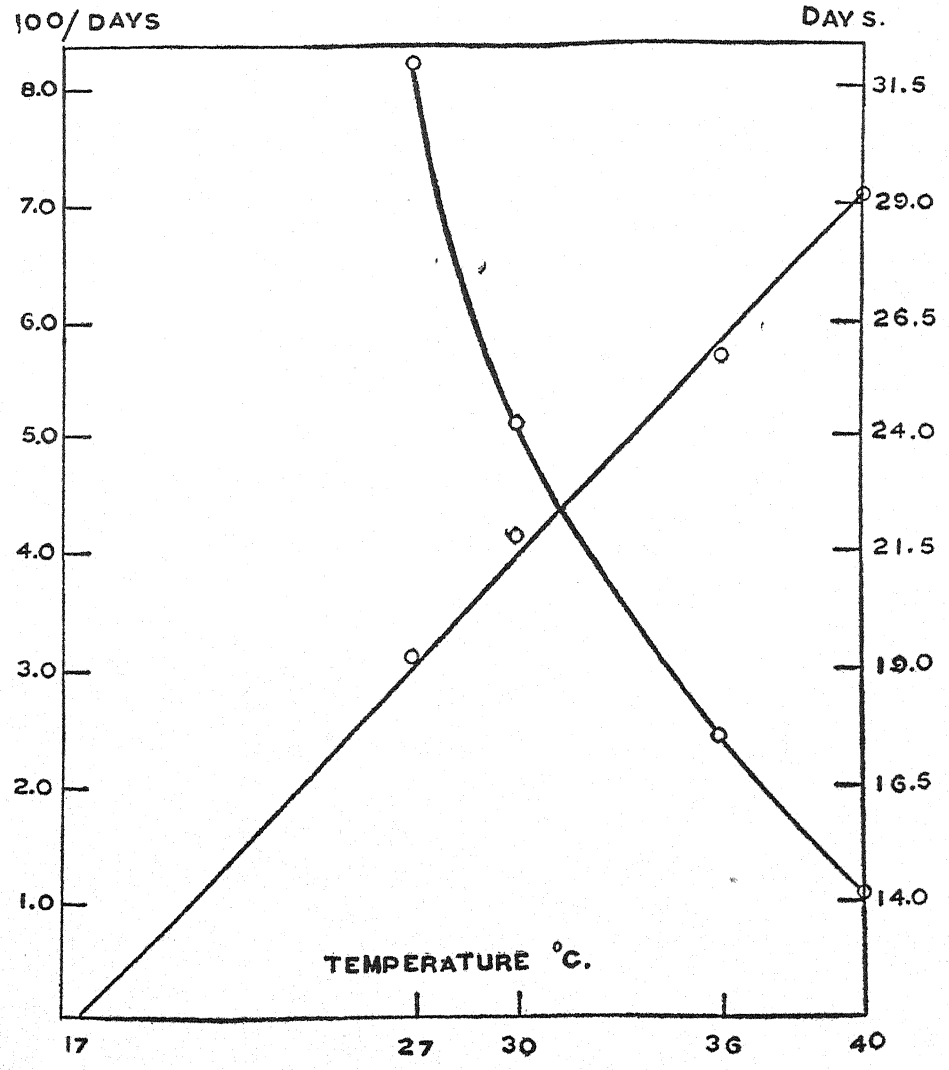

Fig. 1. Time-temperature and temperature-rate curves of pre-oviposition period of *Schistocerca gregaria* adults.

The influence of temperature on the pre-oviposition period is quite significant. The higher the temperature, within certain limits, the shorter the pre-copulation and pre-oviposition period. The shortest period recorded has been thirteen days at 36°C., and 40°C. At 27°C., however, it was thirty-two days. At one and the same constant temperature the period varies from individual to individual. At 40°C., it varied from thirteen to seventeen days, at 36°C., from thirteen to twenty-three days, at 30°C., from seventeen to twenty-eight days and at 27°C., it was thirty-two days.

II. Pre-oviposition period at a temperature varying between 28°C. and 32°C.

Some of the breeding cages were kept in an artificially heated hot house, the temperature of which was maintained between 28°C. and 32°C. Under these conditions the pre-oviposition period varied from fifteen to twenty-eight days (Table II). These results agree with those above stated.

Table II

Pre-oviposition period in the hot house

(Temperature varying from 28-32°C.)

Previous history: Hoppers of V stage were collected from a swarm during 1930 and bred in the laboratory; their progeny was used in these experiments.

(1931 & 1932)

Reference No.	Date of acquiring wings	Date of 1st oviposition	*Pre-oviposition period (days)
L_1	24th Sept.	18th Oct.	24
L_6	24th Sept.	17th Oct.	23
L_9	24th Sept.	16th Oct.	22
L_{17}	24th Sept.	16th Oct.	22
L_{36}	24th Sept.	26th Oct.	32
L_{38}	26th Dec.	20th Jan.	25
L_{39}	3rd Jan.	18th Jan.	15
L_{49}	5th Jan.	26th Jan.	21
		Average	24·2

III. Pre-oviposition period of the over-wintered adults at laboratory temperature

The adults, bred in the laboratory, from hoppers collected from a swarm and which had acquired wings in October 1930, were kept in cages in a large laboratory, during the winter 1930-31. The earliest oviposition occurred in the beginning of February. The average duration of the pre-oviposition period was 131 days, and the longest pre-oviposition period was almost seven months. The temperature of this laboratory remained below 18°C. (the developmental zero) during December and January. Oviposition commenced in the beginning of the spring when the maximum temperature rose to about 21°C. (Table III). If the temperature remains low over a longer period, it is not unlikely that the pre-oviposition period may be as long as ten months, as stated by Bodenheimer [1929]. These observations represent, to a very large extent, what happens under natural conditions. The individuals acquiring wings in the beginning of winter over-winter as adults.

TABLE III

Pre-oviposition period of the over-wintered brood at laboratory temperature

Previous history: V stage hoppers collected from a swarm.

Reference No.	Date of acquiring wings (1930)	Date of 1st oviposition (1931)	Pre-oviposition period (days)
39 A	16th Aug. to 6th Sept.	24th Feb.	171—192
39 B	Do.	17th March	192—213
47 A	2nd to 7th Oct.	23rd Feb.	139—144
47 B	Do.	27th Feb.	143—148
48 A	Do.	18th Feb.	134—139
41	16th Oct. to 3rd Nov.	26th Feb.	115—133
46 B	Do.	7th March	124—142
40 A	16th Oct. to 24th Nov.	15th March	111—150
40 B	Do.	19th Feb.	87—126
35	17th Oct.	30th Jan.	105
	18th Oct.	29th Jan.	103
13	Do.	10th Feb.	115
18 A	20th Oct.	27th Jan.	99
18 B	Do.	11th Feb.	111
49 A	27th to 31st Oct.	3rd March	123—127
50 B	Do.	19th March	139—143
51 B	Do.	15th March	135—139
53	Do.	22nd March	142—146
54	Do.	24th March	144—148
		Average	133

IV. Pre-oviposition period at laboratory temperature during April-July

During April to July the temperature of the laboratory, which served as our main rearing room, varied from 28° to 32°C., and the range of the pre-oviposition period was sixteen to thirty-two days (Table IV). These observations confirm those in the hot house (see p. 193.).

Table IV

Pre-oviposition period at room temperature from April to July 1931 (Temp. 28-32 C.)

Previous history: Hoppers were bred in cages at room temperature.

Reference No.	Date of acquiring wings	Date of 1st oviposition	Pre-oviposition period (days)
N 7 b2	2nd April	4th May	32
N 11	5th to 9th April	9th May	30—34
I 9	27th to 30th April.	26th May	26—29
I 9	27th to 30th April.	28th May	28—31
I 10	5th to 8th May	30th May	22—25
I 12	5th to 10th May	30th May	20—25
I 12	5th to 10th May	30th May	20—25
I 7	5th to 10th May	28th May	18—23
I 11	5th to 12th May	28th May	16—23
I 11	5th to 12th May	29th May	17—24
R 43 d	8th to 13th May	4th June	22—27
I 14	26th to 29th June.	28th July	29—32
Q 1	11th to 15th July.	7th August	23—27
		Average	25·4

The observations given above make it abundantly clear that in the adult stage of the Desert Locust there is no diapause, in the true sense; in other words the rythm of development is not fixed, but is directly dependent on temperature, and perhaps also on other environmental conditions. By exposing adults to a temperature of 36° to 40°C., the pre-copulation and pre-oviposition period was reduced to thirteen days or so, and by keeping insects at a lower temperature it was prolonged to 141 days and over.

There is abundant evidence that in nature, in the Punjab, there are two distinct broods of the Desert Locust. The audlts which emerge during September to November, over-winter in this stage, and begin to copulate and oviposit from February onwards. In certain warmer parts of India these processes may start earlier. As stated above the earliest recorded date of oviposition for the Punjab is 7th February. In the case of this over-wintering brood the pre-oviposition period may be as long as seven months. The adults that develop from the progeny of these over-wintered adults acquire wings from April onward, and their pre-oviposition period is not very long as copulation and egg laying start in July. April to September are the months of high temperature and during October to February the temperature is low. Hence it is evident that it is mainly temperature that controls the pre-copulation and pre-oviposition period, or in other words, the sexual maturity of the adults.

It is also evident that there must be individual differences under the same conditions of temperature, since such factors as morphological and physiological individual variations, food supply, etc., must operate.

3. Inter-oviposition period

Schistocerca gregaria, as is well known, lays a number of egg-pods, at intervals. The interval between two successive ovipositions varies. Regnier [1926] observed that the females oviposited at intervals of fifteen to twenty days. From his field observations, Bodenheimer [1929] recorded two ovipositions by the same swarm: one at the end of February and the other in the beginning of April. Ballard and others [1932] give one day as the minimum, thirty-nine days as the maximum and five to seven days as the normal interval between two successive ovipositions.

I. Inter-oviposition period of females kept at constant temperatures (Table V)

The observations made at constant temperatures show that the minimum inter-oviposition period is three days at 40°, 36° and 30°C. The variation is three to seven days at 40°C. (average 4·8 days); three to seven days at 36°C. (average 4·6 days), and three to twenty-four days at 30°C. (average 9·3 days), and a single observation at 27°C gave the inter-oviposition period as twelve days.

TABLE V

Inter-oviposition period at constant temperatures

Previous history: Hoppers bred crowded in thermostats at the same temperature to which adults were exposed.

Reference No.	Temperature	Dates of oviposition					Inter-oviposition period (days)
		I	II	III	IV	V	
				(*1932*)			
40 I A2 .	40°C. .	27th Feb.	5th March	10th March	...	...	7, 5
40 II A1 .	Do. .	1st April	4th April	8th April	...	...	
40 II A1 .	Do. .	8th April	13th April	...	...	...	3, 4
						Average .	4·8
				(*1931 & 1932*)			
N 6 . .	36°C. .	30th March	2nd April	5th April	...	...	3, 3
N 2 . .	Do. .	31st March	7th April	...	...	...	7
N 1 . .	Do. .	1st April	5th April	...	...	...	4
N 3 . .	Do. .	1st April	4th April	...	...	...	3
I 2 . .	Do. .	2nd June	7th June	...	...	...	5
III 2 . .	Do. .	4th Aug.	11th Aug.	...	...	...	7
A 1 . .	Do. .	18th Oct.	24th Oct.	...	...	...	6
N 2 . .	Do. .	18th Oct.	24th Oct.	...	...	...	6
L 61 . .	Do. .	5th Feb.	8th Feb.	12th Feb.	...	...	3, 4
2 . . .	Do. .	5th Feb.	9th Feb.	13th Feb.	...	...	4, 4
1 . . .	Do. .	7th Feb.	11th Feb.	16th Feb.	...	...	4, 5
3 . . .	Do. .	8th March	12th March	18th March	...	...	4, 6
						Average .	4·6
				(*1932*)			
29 B . .	30°C. .	23rd Jan.	27th Jan.	31st Jan.	6th Feb.	...	4, 4, 6
29 A . .	Do. .	27th Jan.	8th Feb.	23rd Feb.	...	...	12, 15
30 B . .	Do. .	5th Feb.	13th Feb.	23rd Feb.	6th March	16th March	8, 10, 12, 10
30 A . .	Do. .	5th Feb.	10th Feb.	16th Feb.	5th March	...	5, 6, 18
29 C . .	Do. .	7th Feb.	19th Feb.	14th March	17th March	...	12, 24, 3
						Average .	9·3
				(*1932*)			
L 66 . .	27°C. .	19th Feb.	2nd March	...	...	...	12
						Average .	12

II. Inter-oviposition period of adults kept in the hot house at a temperature varying between 28 and 32°C.

In the adults kept in the hot house, the temperature of which was maintained at 28 to 32°C., the inter-oviposition period varied from three to twenty-eight days, the average being 10·2 days (Table VI). It is interesting to note that this agrees fairly closely with the average for 30°C. constant.

Table VI

Inter-oviposition period in the hot house (Temperature 28-32°C.) (1931 & 1932)

Previous history: V stage hoppers collected from swarms and bred in the laboratory.

Reference No.	Dates of oviposition				Inter-oviposition period (days)
	1st	2nd	3rd	4th	
L 9 . .	16th Oct. .	2nd Nov. .	30th Nov.	16th Dec.	17, 28, 16
L 14 . .	12th Nov.	16th Nov.	24th Nov.	28th Nov.	4, 8, 4
L 21 . .	16th Nov.	8th Dec.	12th Dec.	. .	22, 10
L 8 . .	24th Nov.	1st Dec.	5th Dec. .	8th Dec. .	7, 4, 3
L 53 . .	4th Feb.	12th Feb.	16th Feb.	24th Feb.	8, 4, 8
				Average .	10·2

III. Inter-oviposition period at laboratory temperature during different months of the year

In Table VII are given data concerning the oviposition of the females which were kept inside the laboratory, except during February and March when they were exposed to the sun for a few hours every day. It will be seen that from April to August, when the daily average temperature ranged from 30° to 34°C., the inter-oviposition period varied mostly from five to seven days, while in February and March when the temperature in the laboratory remained below 30°C., the inter-oviposition period varied from ten to thirty days in February and five to thirty-five days in March. Thus while the longest inter-oviposition period during April to August was only fourteen days, it was thirty-five days during February and March.

TABLE

Inter-oviposition period

Reference No.	Previous history	Dates of oviposition. 1st	2nd	3rd	4th	5th	6th
A	Fifth stage hoppers collected from a swarm and bred in the laboratory.	27th Jan.	18th Feb.	4th March	15th March	21st March	31st March
18 No. 8		29th Jan.	28th Feb.	4th April	13th April	...	...
35		30th Jan.	12th Feb.	26th Feb.	8th March	19th March	24th March
No. 13		10th Feb.	26th Feb.	11th March	29th March	...	...
18 B		11th Feb.	6th March	?	?	24th March	...
48 A		18th Feb.	12th March	24th March	29th March	...	...
40 B		19th Feb.	7th March	27th March	3rd April	17th April	...
47 A		23rd Feb.	14th March	24th March	1st April	6th April	10th April
39 A		24th Feb.	9th March	19th March	27th March	7th April	...
41		26th Feb.	9th March	19th March	29th March	12th April	...
47 B		27th Feb.	14th March	21st March	26th March	1st April	6th April
46 B		7th March	16th March	27th March	...	...	...
51 B		15th March	29th March	3rd April	...	...	...
40 A		15th March	3rd April	9th April	...	...	...
39 B		17th March	23rd March	28th March	3rd April	9th March	...
50 B		19th March	26th March	6th April	...	...	...
53		22nd March	27th March	4th April	...	...	...
54		24th March	31st March	4th April	...	...	...
I 9A	Hoppers bred crowded in the laboratory from the very beginning.	26th May	2nd June	...	...	...	...
I 7		28th May	5th June	...	...	...	...
I 11		29th May	5th June	...	...	...	...
I 12		30th May	3rd June	...	...	...	...
I 14		24th June	28th June	6th July	10th July	...	...
Q 14		11th July	19th July	...	...	...	...
Q 4		23rd July	28th July	...	...	...	...
I 11		3rd August	8th August	...	...	...	...
I 15		7th August	15th August.	...	...	...	...

VII

at room temperature (1931)

				Inter-oviposition period								
7th	8th	9th	10th	1	2	3	4	5	6	7	8	9
6th April .	...	...	...	22	14	11	6	10	6	...	...	...
...	...	...	...	30	35	9	...	...	...	...	...	...
28th March .	3rd April .	8th April .	14th April .	13	14	10	11	5	4	6	5	6
...	...	...	...	16	13	18	...	...	...	...	...	...
...	...	...	...	23	...	...	...	...	...	...	...	...
...	...	...	...	22	12	5	...	...	...	...	...	...
...	...	...	...	16	20	7	14	...	...	...	...	...
16th April	...	...	...	19	10	8	5	4	6	...	...	...
...	...	...	...	13	10	8	11	...	...	...	...	...
...	...	...	...	11	10	10	14	...	...	...	...	...
11th April .	...	...	...	15	7	5	5	5	5	...	...	...
...	...	...	...	9	11	...	...	...	...	...	...	...
...	...	...	...	14	5	...	...	...	...	...	...	...
...	...	...	...	19	6	...	...	...	...	...	...	...
...	...	...	...	6	5	6	6	...	...	...	...	...
...	...	...	...	7	11	...	...	...	...	...	...	...
...	...	...	...	5	8	...	...	...	...	...	...	...
...	...	...	...	7	4	...	...	...	...	...	...	...
...	...	...	...	7	...	...	...	...	...	...	...	...
...	...	...	...	8	...	...	...	...	...	...	...	...
...	...	...	...	7	...	...	...	...	...	...	...	...
...	...	...	...	4	...	...	...	...	...	...	...	...
...	...	...	...	4	8	4	...	...	...	...	...	...
...	...	...	...	8	...	...	...	...	...	...	...	...
...	...	...	...	5	...	...	...	...	...	...	...	...
...	...	...	...	5	...	...	...	...	...	...	...	...
...	...	...	...	8	...	...	...	...	...	...	...	...

In Table VIII the data of Table VII have been summarised under separate months, and the conclusions mentioned above find full support. As temperature increases progressively from month to month the inter-oviposition period of the Desert Locust decreases. It may, however, be stated that in the Punjab, under natural conditions, eggs are very rarely laid during May and June, oviposition in the over-wintered brood starts in February and in the case of the spring brood, in July.

TABLE VIII

Inter-oviposition period (days) during different months (1931) at room temperature.

Month	Average room temperature (°C.)	Individual observations on inter-oviposition period	Average
February	12·2 to 14·5	30, 16, 22, 14, 23, 13, 14, 10, 13, 16, 11, 22.	17·0
March	19·3 to 22·0	35, 13, 18, 11, 6, 10, 11, 5, 4, 10, 8, 6, 5, 19, 20, 10, 10, 9, 19, 10, 15, 7, 5, 6, 12, 5, 7, 11, 14, 5, 8, 7.	10·6
April	26·6 to 30·0	9, 6, 6, 5, 6, 11, 6, 6, 6, 7, 14, 5, 4, 6, 5, 5, 8, 11, 5, 4.	6·8
May	28·2 to 33·3	7, 8	7·5
June	31·2 to 34·2	4, 8, 4, 7	5·7
July	29·5 to 32·0	4, 5, 8	5·7
August	32·7 to 33·7	5, 8	6·5

IV. Winter intervening between two ovipositions

We were exceedingly fortunate in obtaining two most interesting records of oviposition by the same females, once before winter and again after winter (Table IX). These two females acquired wings in August-September, and laid their first cluster of eggs in October 1930, and after a period of 90 days and 125 days, laid their second clusters in January and February 1931. These two instances further indicate the influence of low temperature in prolonging the inter-oviposition period. Whether a similar phenomenon can occur in nature is worth investigating. Once again this observation proves that there is no diapause in the adult stage and that besides other factors, temperature is responsible for the control of processes connected with oviposition.

Table IX

Inter-oviposition period prolonged by the intervention of winter

Experiment No.	1st oviposition	2nd oviposition	Inter-oviposition period (days)
1	15th October 1930 .	17th February 1931	125
2	25th ,, ,, .	23rd January ,, .	90

From what has been stated above it is quite evident that in the same individual at the same temperature the period between different ovipositions may vary, as for instance from three to twenty-four days at 30°C. Undoubtedly the inter-oviposition period is determined by other factors, internal and external, although temperature is not without marked influence. It is evident that in case of a fall in temperature this period is lengthened, while at suitable temperatures successive batches of eggs may be laid at short intervals.

4. Number of ovipositions by a female

From their observations in cages Kunckel and Regnier admitted eleven successive ovipositions by the same female. Vosseler [1905] regards two ovipositions as not impossible. Bodenheimer was inclined to admit two ovipositions by a swarm in nature. Ballard and associates [1932] give six batches as the maximum.

An examination of Table V shows that at high temperatures, *i.e.*, 36°C. and 40°C., the total oviposition period was much shortened and the maximum number of egg clusters was only three, which were all laid within a fortnight. At 30°C., however, five clusters were recorded which were laid in about 1½ months.

Numerous observations were made at room temperature (Table VI). The maximum number of egg-pods laid by the same female, that we have recorded, was eleven, from an over-wintered individual, which finished its oviposition before the laboratory temperature rose above 30°C. From June to August, however, when the laboratory temperature usually remained above 30°C., the number of oviposition was greatly reduced, the maximum being three. This confirms the results obtained in the constant temperature incubators.

5. Oviposition

I. Oviposition at high soil temperature

There is no previous record of observations on the influence of soil temperature on the behaviour of female in boring into the soil and ovipositing in it. To find out the highest soil temperature at which oviposition could take place the following experiment was performed:—

A rearing cage (Fig. 2) with three storeys was prepared. The uppermost portion (A) was 8 in. × 8 in. × 10 in., three sides and the top of which were of wire gauze. The fourth side was a glass door. The locusts were kept in this portion. The middle portion (B) had the same dimensions as the upper portion but all the four sides were enclosed with ply-wood sheets; in one of the sides holes were provided for inserting the thermometers (th). This portion was filled with sandy soil(s), for purposes of oviposition. The lowermost portion (C) was 5·5 in. × 5·5 in. × 7 in. and was made of 'tin'. One of its sides had two holes. The upper one (a) for the escape of steam and for replenishing the water lost through evaporation, and the lower one (b) carried a cork and a glass tubing which served to indicate the level of water in the tin.

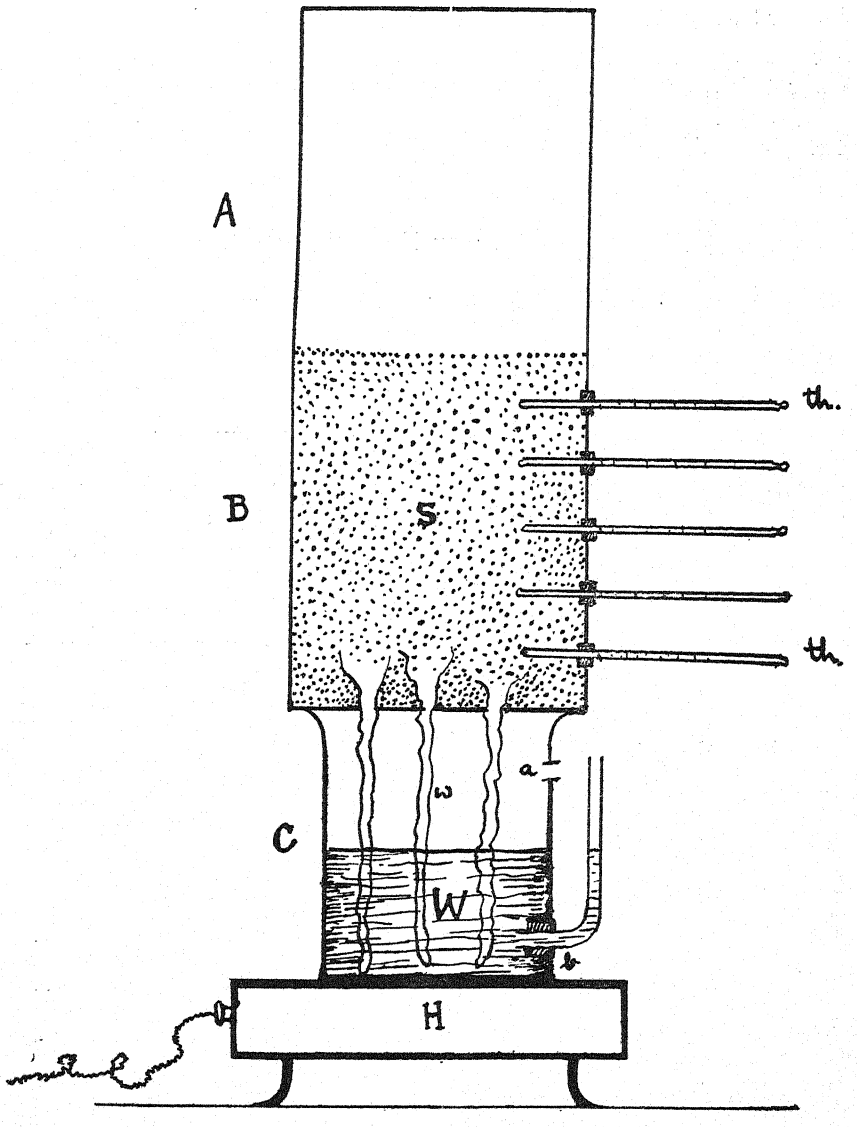

Fig. 2. Rearing cage.

Between the lower and the middle portions there was a wire-gauze partition supported by wooden frame-work. The soil in the middle portion was placed in constant contact with the water(W) in the lowest portion by means of cotton wicks(w). As the water in the soil evaporated, the loss of moisture was made good by water sucked up by capillary action through the wicks. The water was

heated by an electric heater(H) placed at the bottom of the cage. Thus the soil was maintained at an appreciably higher temperature than the top portion A.

It was found that a female could bore into the soil when the surface temperature was 42°C., and the soil temperature at the depth of three inches, where the egg-pod was laid, was 48°C. None of these eggs, however, hatched out (Table X). It would be interesting to perform further experiments along these lines.

TABLE X

Oviposition at high soil temperature

Date of oviposition (1931)	Depth of bore in inches	Temperature in cage in Centigrade				Soil temperature at the depth of egg-pod at the time of oviposition (°C.)
		Air	Soil surface	Soil at 2·5 in.	Soil at 4·5 in.	
22nd June . . .	2·5	36	42	45·5	55	..
12th August . . .	4	34·5	37·5	42·5	51·5	..
26th „ . . .	4	..	..	44	..	45
21st September . .	..	31·4	32·2	43·4	52·8	44
20th—21st Oct. . .	2·5	28·4	30·8	46·2	55·8	..
23rd Oct. . . .	2·5	32·4	33	40·8	58·4	..
27th Oct. . . .	3·2	29·8	30·4	41·2	51·8	..
18th Nov. . . .	2·5	27·4	31·2	39	46·4	40
21st Nov. . . .	3·5	28·2	30·4	39·8	51·4	42·5
23rd Nov. . . .	3	..	38	45	56	46
26th Nov. . . .	3	25	30	46·5	59	48
28th Nov. . . .	3·2	28	30	41	50	43·5
13th Nov. . . .	2·5	27	30	42	50	42

NOTE.—Eggs did not hatch in any case.

II. Oviposition at low soil temperature

To study the influence of low soil temperature on oviposition the following experiments were performed:—

A wooden cage 24 in. × 24 in. × 12 in. was filled with sandy-loan soil upto six inches. In the centre of this cage a metallic jar about six inches in diameter and six inches in depth was buried in the soil. This was always kept filled with ice. This arrangement gave us a series of soil temperatures ranging from about 0°C., next to the ice jar, to 30°C., in the farthest corners of the cage. The temperature of the soil was recorded by a series of thermometers inserted at regular intervals from the cooling vessel, the bulbs being placed at 3-4 in.— the usual depth at which the eggs were laid. A number of mature pairs was placed in this cage. It was noticed that all the thirteen egg-pods laid were deposited in the four corners of the cage, *i.e.*, farthest from the cooling vessel (Fig. 3). In these places the temperature ranged from 25·5° to 28·8°C. The details of this experiment are given in Table XI.

Table XI

Oviposition at low soil temperature (Figs. 3 & 4).

Date of oviposition (1931)	Distance from centre (inches)	Depth to the bottom of pod (inches)	Temperature at the time of oviposition (°C.)
	Experiment I		
2nd July	10	3·5	..
7th July	9·5	3·25	..
11th July	10·25	3	28
11th July	10·0	..	28·6
11th July	11	..	29
15th July	10·5	3·75	26
17th July	..	..	28
18th July	9·5	2·5	25·5
	Experiment II		
14th Aug.	7	2·7	21·5
14th Aug.	7	3·8	21·0
15th Aug.	7	..	20·0
19th Aug.	6·5	2	23·3

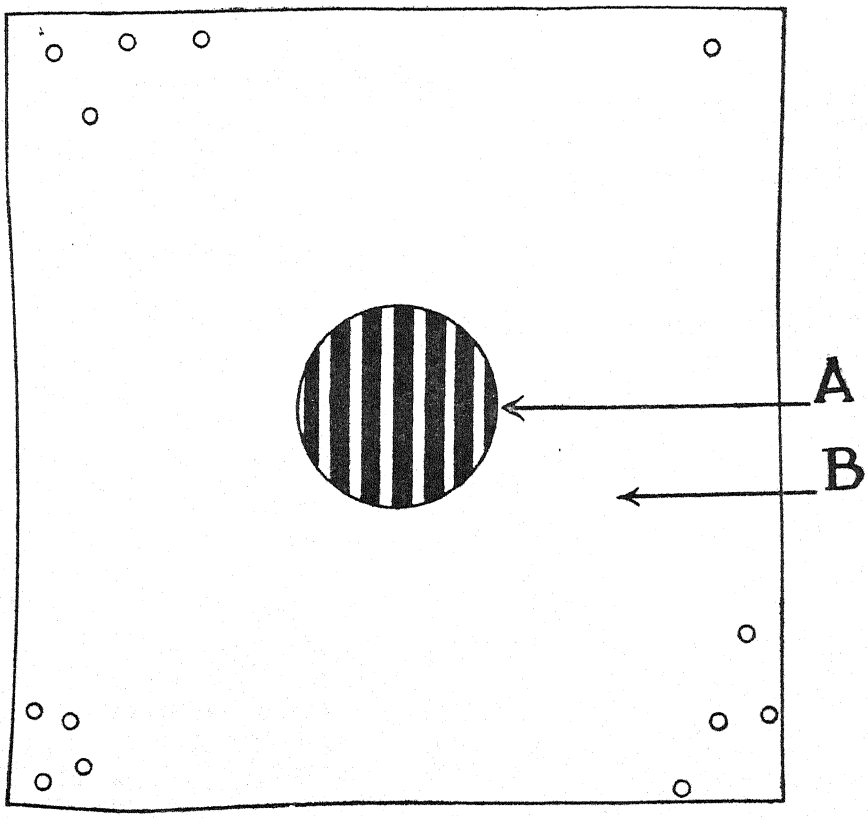

Fig. 3. Cross-section of the cool cage.
A = Cooling vessel, B = Soil available for oviposition.
O = Places where eggs were laid.

This experiment was further modified with a view to force the females to lay eggs at a still lower temperature. With this object access to the more distal parts of the soil was cut off by covering it with a tin sheet. Thus only a circular band of soil was left exposed (Fig. 4). In this case also the eggs were laid farthest from the cooling vessel along the circumference of the soil exposed. The lowest soil temperature at which we obtained eggs was 20°C. This would appear to be the lowest limit, as it approaches the developmental zero of the various stages of this insect. Further observations are, however, necessary to fix the lowest limit definitely.

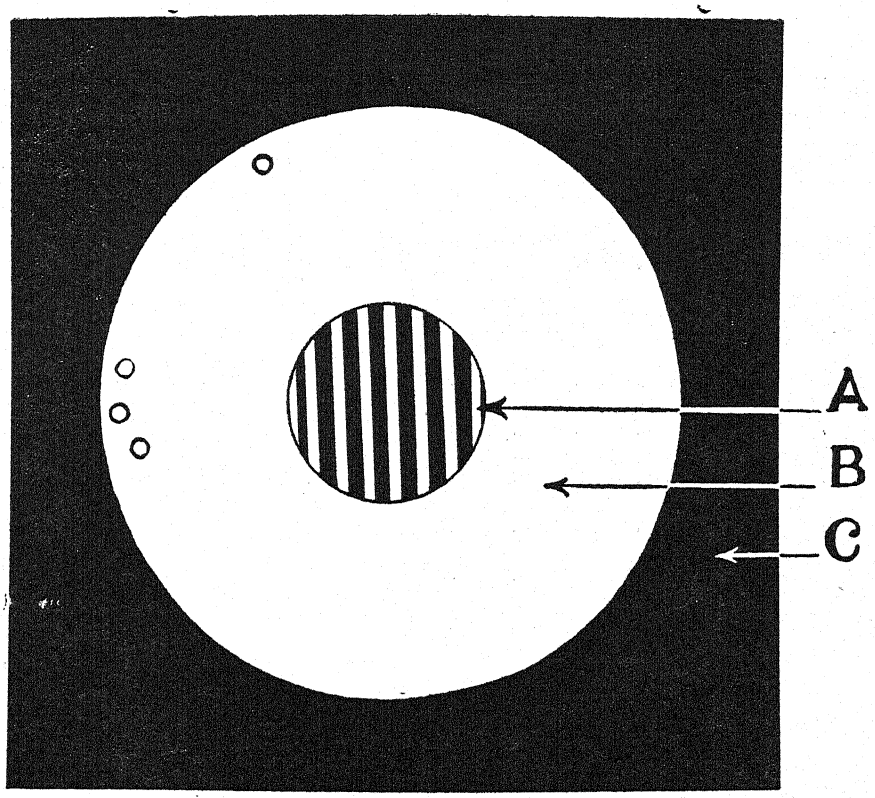

Fig. 4. Cross-section of cool cage (modified).
A = Cooling vessel. B = Soil available for oviposition.
C = Soil covered over with tin sheet.
O = Places where eggs were laid.

6. Incubation

The incubation period for the eggs of *Schistocerca gregaria* has been recorded by various investigators, and the available data are summarised below:—

Investigator	Country	Incubation period
Vosseler	South Africa	16—18 days.
Gough (1915)	Egypt	16—30 days.
Bucher (1915)	Palestine	4 weeks (28 days).
Regnier (1926)	..	16—40 days.
Telenga (1930)	..	3 weeks (21 days).
Bodenheimer (1930)	Palestine	30 days in Feb. and March, 14 days in May.

Observations of various investigators in the Punjab, under natural conditions, place the incubation period at three to four weeks in winter, and a fortnight in summer.

Ballard and others [1932] have given a statement of the incubation period of eggs laid during different months of the year which we quote below:—

Months of oviposition	Average incubation period (days)	Month of hatching
March	43	April—May
April	31	April—May
May	21	May—June
August	23	August—September
September	34	Sept.-Oct.-November
November (without winter diapause)	19	November
November (Winter egg diapause)	106	February—March
December (Winter egg diapause)	95	March
January (Winter egg diapause)	79	March—April
February (Winter egg diapause)	67	April

Unfortunately no mention is made as to whether the cages were kept in a room or in the open. Nor is there any information concerning the temperature of the soil in which the eggs remained. It is, however, evident that these eggs remained under different conditions of temperature, and perhaps also of soil moisture.

It is known that soil-moisture deficiency prolongs the incubation period and in one of our experiments we have prolonged it to seventy days, though under the same conditions of temperature the normal incubation period was found to be only twelve days.

I. Incubation of eggs at constant temperature

The incubation of the eggs of the Desert Locust has been studied under constant temperatures. For this purpose freshly laid eggs were removed from the oviposition cages, washed in distilled water and placed in sterilized moist sandy soil, in glass tubes. These tubes were kept in incubators at different temperatures. In some cases the temperature was constant, while in certain other cases,

such as the multiple incubator, it varied within a few degrees. Similarly there was variation in the temperature of the cool cage described above. Our data of incubation at different temperatures are not complete: we have had numerous failures in these experiments because of the fungi and nematodes attacking the eggs.

(*a*) *Threshold of development*

To determine the minimum temperature at which development of *Schistocerca gregaria* eggs could take place the following experiments were performed. Two batches of eggs laid on 28th Feb. 1934, at eleven and thirteen hours, by two different females, were left in the soil of the cage at room temperature (18-21°C.) till twelve hours the next day. On 1st March, 1934 they were taken out, washed with water and placed in dried sterilized soil, which was then moistened with sterilized water. Five eggs were placed in each tube and eleven tubes were set up. The mouths of the tubes were sealed with wax-paper and wax. These tubes were placed in different compartments of the multiple incubator at sixteen hours on 1st March, 1934. The incubator worked satisfactorily till 4th April, 1934, when the low-temperature incubator went out of order, and all the tubes in which hatching had not occurred were transferred to a constant temperature thermostat working at 35°C. The details of this experiments are given in Table XII.

TABLE XII

First set: the threshold of development of Schistocerca gregaria *eggs*

Reference No. L414/28th Feb. 1934. Eggs laid on 28th Feb. 1934. Experiment set up on 1st Mar. 1934.

Tube No.	Initial treatment			Subsequent treatment		Incubation period* (days)	No. of eggs	No. of hoppers	Remarks
	Temperature	Date of hatching	Incubation period (Days)	Date of transferring to 35°C.	Date of hatching				
1	20—21°C. .	2nd April .	32+1	...	...	32+1	5	4	Soil examined on 4th April 1934. 4 hatched eggs and one rotten. ...
2	„ .	2nd April .	32+1	...	...	32+1	5	5	
3	„ .	2nd April .	31+1	...	...	31+1	5	1	Soil examined on 4th April 1934. One hatched egg and 4 plump yellowish brown undeveloped eggs ...
4	„ .	1st April .	31+1	...	...	31+1	5	5	
5	18—19°C. .	...	...	4th April .	11th April	34+7+1=42	5	5	...
6	„ .	...	...	„ .	11th April 12th April	34+7+1=42 34+8+1=43	5	3 2	...
7	„ .	...	...	„ .	...	...	5	...	Soil examined on 17th April 1934. Eggs rotten with plenty of nematodes in the soil.
8	„ .	...	...	„ .	...	...	5	...	
9	16—17°C. .	...	...	„ .	15th April	34+11+1=46	6	5	Soil examined on 17th April 1934. Remaining eggs shrivelled. ...
10	„ .	...	...	„ .	15th April 16th April	34+11+1=46 34+12+1=47	6	4 2	
11	„ .	...	...	„ .	15th April 16th April	34+11+1=46 34+12+1=47	6	3 3	...
12	„ .	...	...	„ .	15th April 16th April	34+11+1=46 34+12+1=47	6	5 1	...
13	13°C. (about)	...	...	„ .	15th April 16th April	34+11+1=46 34+12+1=47	5 5	1 4	...
14	„ .	...	...	„ .	15th April	34+11+1=46	5	3	Soil examined on 17th April 1934. Remaining two eggs with full grown dead larvae.
15	„ .	...	...	„ .	15th April 16th April	34+11+1=46 34+12+1=47	7	3 3	Soil examined on 17th April 1934. The remainng egg had rotted.
16	„ .	...	...	„ .	15th April 16th April	34+11+1=46 34+12+1=47	8	3 5	...

* NOTE.—In this column the first figure indicates the number of days the eggs were kept under the initial treatment , the second number indicates the days at the subsequent treatment and the third number, *i.e.*, (1) indicates the period passed at room temperature (18-21°C.) before the experiment.

TABLE XII—*contd.*

Second set: the threshold of development of Schistocerca gregaria *eggs*

Reference No. 416/28th Feb. 1934. Eggs laid on 28th Feb. 1934. Experiment set up on 1st Mar. 1934.

Tube No.	Initial treatment			Subsequent treatment			No. of eggs	No. of hoppers	Remarks
	Temperature (°C.)	Date of hatching	Incubation period (days)	Date of transferring to 35°C.	Date of hatching	Incubation period* (days)			
16	20—21°	1st April	31+1	...	...	31+1	5	5	...
15	,,	1st April	31+1	...	...	31+1	5	3	Soil examined on 4th April 1934. Three hatched and two rotten eggs.
14	,,	2nd April	32+1	...	...	32+1	4	4	...
13	,,	1st April	31+1	...	...	31+1	4	3	Soil examined on 4th April 1934. Three hatched eggs only.
12	18—19°	...	...	4th April	10th April	34+6+1=41	6	5	Soil examined on 17th April 1934. 5 hatched eggs and one egg which looked plump and yellowish without embryo.
11	,,	...	...	,,	11th April	34+7+1=42	4	4	...
10	,,	...	...	,,	...	...	4	...	Soil examined on 17th April 1934. The eggs contained full-grown but dead embryo.
9	,,	...	...	,,	12th April	34+8+1=43	5	5	...
8	16—17°	...	...	4th April	15th April	34+11+1=46	5	4	Soil examined on 17th April 1934. 4 hatched eggs and one rotten egg.
7	,,	...	...	,,	15th April	34+11+1=46	5	4	Soil examined on 17th April 1934. 4 hatched eggs and one plump but undeveloped egg.
6	,,	...	...	,,	16th April	34+12+1=47	4	4	...
5	,,	...	...	,,	17th April	34+13+1=48	5	5	...
4	13° (about)	...	...	4th April	15th April	34+11+1=46	5	5	...
3		...	...	,,	15th April	34+11+1=46	5	5	...
2		...	...	,,	15th April	34+12+1=47	6	4	Soil examined on 17th April 1934. 6 hatched eggs and 2 eggs with vermiform larvae that could not come above the soil.
1		...	...	,,	15th April 16th April	34+11+1=46 34+12+1=47	9 ...	3 6	...

* NOTE.—In this column the first figure indicates the number of days the eggs underwent the initial treatment, the second number indicates the number of days at the subsequent treatment and the third number *i.e.* (1) indicates the period passed at room temperature (18-21°C.) before the experiment.

Besides the above-mentioned two sets of experiments, three more sets on similar lines were arranged. Unfortunately none of the eggs in them, including the control, hatched.

At a temperature of 20-21°C. the incubation period was thirty-one to thirty-two days (*plus* one day at room temperature, 18-21°C.). At the other three temperatures, *i.e.*, 18°-19°C., 16°-17°C., and approximately 13°C., the eggs remained for thirty-four days without hatching. After this period the tubes were transferred to 35°C. It is interesting to note that in the case of tubes kept at 18°-19°C., the eggs hatched out after seven to eight days-incubation at 35°C., showing that some development had taken place at 18°-19°C. In case of the tubes at 16°-17°C., and 13°C., a period of eleven to thirteen days incubation at 35°C., was necessary for hatching. From this it is evident that no development had taken place at these low temperatures. We thus conclude that the threshold of development is somewhere between 17° and 18°C.

At 35°C., the normal period of incubation is twelve days (Table XIII). The additional time taken by tubes kept for thirty-four days at 18°-19°C., is equal to 7·2 days at 35°C. One may conclude therefore that about forty per cent of the development had taken place at 18°-19°C., before these eggs were transferred to 35°C. From this it may be calculated that the incubation period at 18°-19°or 18·5°C., would be:—

$$34 \times 5/2 = 85 \text{ days.}$$

(*b*) *Incubation period at constant temperatures*

The results of experiments on the incubation period at constant temperatures, controlled by electric thermostat, are given in Table XIII(A), and those at temperatures controlled by multiple incubator with a variation of approximately 1·5°C. are given in Table XIII(B). Time-temperature and temperature-rate curves based on Table XIII are shown in Fig. 5. The observations carried out in the multiple incubator are indicated by cross '×' on the index line. It will be noticed that while these crosses do not lie exactly on the index line, they follow more or less the same course, the variation being due to the less constant temperatures in this case. From these data we arrive at the following general conclusions:—

Below 20°C., we did not get any hatching although some development went on even at 18-19°C., as indicated by experiments on the threshold of development. The theoretical threshold of development (Fig. 5) lies at 16°C.

At a temperature varying between 20° and 21°C., the incubation period was thirty-one to thirty-two (+1) days, between 20·5° and 23·8°C., it was twenty-eight to thirty-one days, and between 25·5°C. and 28·8°C., the incubation period was eighteen to twenty-one days. At 33°C. and 35°C. constant it was twelve to thirteen days and at 40°C. it was about eleven days. The shortest incubation period recorded was nine days at a temperature varying between 43° and 46°C., in the multiple incubator. It may be stated that our multiple incubator did not work very satisfactorily and the temperature varied considerably. Ordinarily we did not get any hatching above 43°C., and the higher temperature of 46°C., in the above experiment must have been for a very short duration.

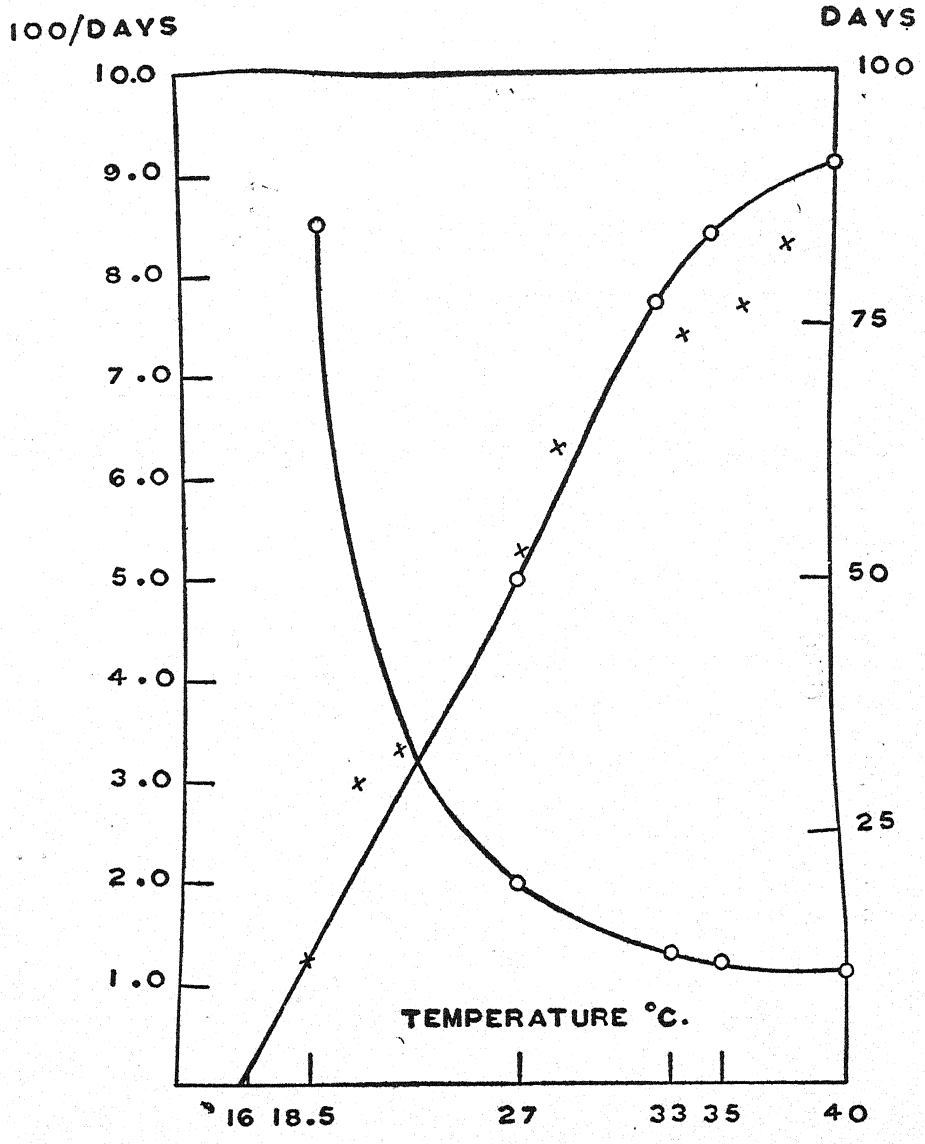

Fig. 5. Time temperature and temperature-rate curves of incubation period of *Schistocerca gregaria* eggs.

O = Observations from electric thermostat.

× = Observations from multiple incubator and the cool cage.

TABLE XIII

Incubation period at constant temperatures

(1931)

Reference No.	Temp. (°C.)	Date of oviposition	Date of hatching	Incubation period (days)	Mean period (days)	No. of hoppers hatched	Remarks
			A.—Electric thermostat with a variation of about ±0·5°C.				
....	27	11th Dec.	31st Dec.	20	20·0	7	Eggs kept in moist soil in tube
Q31 (*b*) I	33	20th Aug.	2nd Sept.	13	..	..	Eggs exposed to 100 per cent humidity
Q31 (*a*) I	33	20th Aug.	2nd Sept.	13	..	..	Ditto
Q31 (*a*) III	33	26th Aug.	7th Sept.	12	13·0	3	Eggs kept in moist soil in tube
Q31 (*a*) I	35	20th Aug.	2nd Sept.	13	..	..	Eggs exposed to 100 per cent humidity
Q31 (*b*) I	35	20th Aug.	1st Sept.	12	..	..	Ditto
Q31 (*b*) III	35	26th Aug.	6th Sept.	11	..	4	Eggs kept in moist loam soil in tube
L9	35	16th Dec.	28th Dec.	12	12·0	5	Ditto
Q31 (*a*) I	37	20th Aug.	1st Sept.	12	..	..	Eggs exposed to 100 per cent humidity
Q31 (*b*) I	37	20th Aug.	1st Sept.	12	..	..	Ditto
Q35 II	37	24th Aug.	6th Sept.	13	..	..	Ditto
Q31 (*c*) III	37	26th Aug.	6th Sept.	11	..	4	Eggs kept in moist loam soil in tube
Q31 (*d*) III	37	26th Aug.	7th Sept.	12	12·0	4	Ditto
L13	40	13th Dec.	24th Dec.	11	11·0	8	Ditto

TABLE XIII—*contd.*

Incubation period at constant temperatures

Reference No.	Temperature (°C.)	Date of oviposition	Date of hatching	Incubation period (days)	Mean period (days)	No. of hoppers hatched	No. of eggs
B.—In multiple incubator and cool cage (the variation being about ± 1·5°C.)							
Cool Cage . .	22·2 ± 1·6 .	14th Aug. .	11th-12th Sep	28—29	..	35	Full egg pod
,, . .	22·2 ± 1·6	14th ,, .	13th Sep. .	30	..	..	,,
,, . .	22·2 ± 1·6	15th ,, .	13th ,, .	29	..	..	,,
,, . .	22·2 ± 1·6	19th ,, .	20th ,, .	31	29·0	..	,,
,, . .	27·2 ± 1·6	2nd July .	22nd July .	20	..	30	,,
,, . .	27·2 ± 1·6	7th ,, .	28th ,, .	21	..	17	,,
,, . .	27·2 ± 1·6	11th ,, .	29th ,, .	18	..	82	,,
,, . .	27·2 ± 1·6	11th ,, .	30th ,, .	19	..	71	,,
,, . .	27·2 ± 1·6	11th ,, .	30th ,, .	19	..	71	,,
,, . .	27·2 ± 1·6	15th ,, .	2nd Aug. .	18	..	52	,,
,, . .	27·2 ± 1·6	17th ,, .	4th ,, .	18	..	33	,,
,, . .	27·2 ± 1·6	18th ,, .	7th July .	20	19·0	48	,,
9 . .	29·0 ± 1·0	16th Nov. .	2nd Dec. .	16	16·0	8	10
L2 . .	34·0 ± 1·0	5th Dec. .	20th ,, .	15	..	8	13
L8 . .	34·0 ± 1·0	5th ,, .	21st ,, .	16	..	3	13
L19 . .	34·0 ± 1·0	5th ,, .	17th Nov. .	12	14·0	7	10
L2 . .	36·5 ± 1·5	5th ,, .	18th Dec. .	13	..	12	13
L13 . .	36·5 ± 1·5	5th ,, .	18th ,, .	13	13·0	13	13
L19 . .	38·3 ± 1·2	5th Sep. .	17th Sep. .	12	12·0	5	10
L2 . .	42·0 ± 2·0	5th Dec. .	17th Dec. .	12	..	6	13
L3 . .	42·0 ± 2·0	5th ,, .	17th July .	12	12·0	7	13
L19*a* . .	44·5 ± 1·5	5th Nov. .	14th Nov. .	9	..	4	10
L19*b* . .	44·5 ± 1·5	5th ,, .	14th ,, .	9	9·0	6	10
...	18·5 ± 0·5	From the data on threshold of development (Table XII).			85·0	24	24
...	20·5 ± 0·5	Ditto			32·4	30	38

(c) *Vital limits for the eggs*

The eggs exposed to 10-15°C., during the night and above 20°C., during the day hatched out successfully. The low temperature completely inhibits embryonic development, but the embryo is not killed even if the low temperature prevails for several hours. Eggs exposed to a temperature of about 13°C., for thirty-four days retained their viability and when subsequently exposed to 35°C., hatched out in eleven to twelve days, which is the normal incubation period at that temperature. It is not possible to confirm the statement that exposure to low temperature accelerates development [Parker, 1930].

To determine the upper vital limit, one to two days old eggs were removed from the soil in which they had been laid, washed in distilled water, and placed for a definite period on moist sterilised cotton or sand at the required temperature, and then removed and incubated at 30-33°C. (Table XIV). These essentially preliminary experiments show that the eggs kept at 46°-46.5°C., for two hours retained their viability, while those exposed to 47°-47·5°C., for two hours did not develop. Again, an exposure of eigh thours to 46°-46·5°C., killed the eggs. Further observations are necessary to determine definite limits of temperature and duration of exposure.

TABLE XIV

The upper vital limit of temperature for the eggs of Schistocerca gregaria *1931*)

Reference No. L10
Date of oviposition . . . . . 18th Nov. 1931.
Date of removal and experiment . . . 19th Nov. 1931.

Reference No.	Date of oviposition	Date of experiment	No. of eggs	Temp. to which exposed (in °C.)	Period of exposure (hrs.)	No. of hoppers emerged
L8 . .	24th Nov.	26th Nov.	10	48—47·5	24	..
Q1 . .	1st ,, .	2nd Sept. .	4	48·5—47	4	..
L8 . .	24th ,, .	26th Nov.	10	48—47·5	2	..
L10 . .	18th ,, .	19th ,, .	10	47·5—47	8	..
L10 . .	18th ,, .	19th ,, .	10	47·5—47	2	..
L8 . .	24th ,, .	26th ,, .	10	47—46·5	24	..
L8 . .	24th ,, .	26th ,, .	10	47·5—47	2	..
QA7 .	31st Aug. .	2nd Sept. .	4	47—43	10	..
L10 . .	18th Nov.	19th Nov.	10	46·5—46	8	..
L10 . .	18th ,, .	19th ,, .	10	46·5—46	2	5
Q1 .	1st Sep. .	2nd Sep. .	4	46—45	2	3
Q1 . .	1st ,, .	2nd ,, .	4	45—44	2	3
QA7 .	31st Aug. .	2nd ,, .	4	44—43	2	2
QA7 . .	31st ,, .	2nd ,, .	4	42·5-41·5	3	3

11. INCUBATION PERIOD AT ALTERNATING TEMPERATURES

An experiment was conducted in which freshly laid eggs from the same egg cluster, were transferred into moist sandy loam in four different tubes. Tube No. 1 was kept at 37°C., throughout the incubation period. No. 2 was kept for two days at 25°C., and the remaining period at 37°C., tube No. 3 was kept for ten days at temperatures between 33° and 37°C., and the remaining period at 27°C., while tube No. 4 was placed for two days at 20°C., four days at 25°-27°C., and the remaining period at 33°-37°. The results are given in Table XV.

TABLE XV

Effect of alternating temperatures on incubation period

Ref. No. 35. Date of oviposition 23rd August 1931

Tube 1	Tube 2				Tube 3				Tube 4			
	Date	Time	Temp. (°C.)	Duration (hrs.)	Date	Time	Temp. (°C.)	Duration (hrs.)	Date	Time	Temp. (°C.)	Duration (hrs.)
37°C. constant	23rd Aug.	16·0	37	44	23rd Aug. .	16·0	37	92	23rd Aug.	16·8	20	40
	25th „ .	12·0	25	48	27th „ .	12·0	35	72	25th „ .	8·0	25	52
	27th „ .	12·0	37	240	30th „ .	12·0	33	72	27th „ .	12·0	27	48
					2nd Sept. .	12·0	25	120	29th „ .	12·0	33	48
									31st „ .	12·0	35	48
									2nd Sept. .	12·0	37	120
Date of hatching 5th Sep.	6th Sept.				7th Sept.				7th Sept.			
Total incubation period (days)— 13	14				15				15			

The eggs took thirteen, fourteen, fifteen and fifteen days, respectively to hatch.

By arranging the duration (in days) of each tube under each temperature we get the following figures:—

Tube No.	30°—37°C.	25°—27°C.	20°C.	Total incubation period (days)
1	13	..	..	13
2	12	2	..	14
3	10	5	..	15
4	9	4	2	15

III. INCUBATION PERIOD UNDER VARYING TEMPERATURES IN BREEDING CAGES KEPT IN OPEN

To simulate natural conditions two breeding cages 9 in. × 9 in. × 18 in., the lower portion of which contained sandy-loam soil with glass on four sides and the upper portion surrounded by wire-gauze, were placed on the roof of the insectary building, after eggs had been laid. This experiment was conducted during August, 1931. Daily records of soil temperature at the place of egg-pod and air temperature inside the cage at 7 A.M., 12 noon and 6 P.M. were maintained. During day time these cages were exposed to the maximum solar heat and during night to minimum air temperatures. The incubation period in both cases was thirteen days. Field observations during this period showed that the incubation period was about eleven days. It is evident from the record that during certain portion of the day the eggs were exposed to a maximum soil temperature of 43·8°C. (Table XVI).

TABLE XVI

Temperature record (°C.) *in cages kept in the open*

Date (August)	Maximum soil temperature	(Cage No. 1 ; Date of oviposition, 15th August 1931)						Cage No. 2 ; (Date of oviposition, 16th August 1931)					
		7 A.M.		12 NOON		6 P.M.		7 A.M.		12 NOON		6 P.M.	
		Air	Soil	Air	Soil	Air	Soil	Air	Soil	Air	Soil	Air	Soil
16 . .	35·5	..	..	42	..	36	39	..	..	..	..	34·5	36
17 . .	44·4	30	29·5	39·5	41	39·5	43	30	30	39	40	38·5	39·5
18 . .	42·2	29·8	29·2	39·8	40·8	30·5	33·2	29·8	29	38·5	38·2	30	30·2
19 . .	39·4	28·5	27·8	38	39·8	32·2	33·8	28·5	27·8	37·2	38	32·2	32·8
20 . .	41·6	28·6	27·8	39	40·8	38	40·8	28·5	28	38	39·5	37·8	39
21 . .	34·4	26·2	25·2	36·2	37·2	36	38	26·2	25·5	36·2	35·5	36	37
22 . .	41·6	27·0	26·5	38·2	39·2	40·8	41·8	28	27·8	36·5	36	40·2	38·8
23 . .	41·1	28·5	28·0	40·5	41·2	36	39	28·8	29	40	38·5	36	37
24 . .	38·8	28·5	28·5	40·5	40·2	33·5	34·8	28·5	28·8	40	39·5	33·5	35·2
25 . .	43·3	27·2	27	38·8	40·5	37	38·5	27·8	27	38·8	38·5	37	39·5
26 . .	43·8	28·8	28·8	41·2	42·8	39·2	40·5	28·8	28·2	41·0	42·0	39·5	40
27 . .	41·1	26·0	26·8	40·2	39·5	35·2	37·2	26·2	26·4	39·5	37·0	35·8	34·5
28 . .	..	27·2	26·8	37·0	38·0	..	..	27	27·5	36·8	36·5	35·8	35·5

Date of hatching—28th August 1931

Date of hatching—29th August 1931

IV INCUBATION PERIOD AT LOBORATORY TEMPERATURE DURING DIFFERENT MONTHS.

Observations on the dates of oviposition and hatching were recorded in a very large number of cases kept at laboratory temperature. The results are presented in Table XVII, and the temperature record in Table XVIII.

TABLE XVII

Incubation period (days) at room temperature during different months (1931)

Previous History: Hoppers of V stage collected from a swarm and adults bred one pair in each cage.

January—March			February—April			March—April			April—May			May—June			June—July			July—August			August—September			September			October—November		
Date of		Incubation period	Date of		Incubation period	Date of		Incubation period	Date of		Incubation period	Date of		Incubation period	Date of		Incubation period	Date of		Incubation period	Date of		Incubation period	Date of		Incubation period	Date of		Incubation period
Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching		Oviposition	Hatching	
9th Jan.	14th Feb.	36	2nd Feb.	1st Apl.	58	2nd Mar.	27th Mar.	25	1st Apl.	19th Apl.	18	4th May	24th May	20	2nd June	21st June	19	10th July	20th July	10	3rd Aug.	15th Aug.	12	4th Sep.	20th Sep.	16	18th Oct.	2nd Nov.	15
			3rd ,,	22nd Mar.	47										2nd ,,	15th	13	28th ,,	10th Aug.	13	7th ,,	20th ,,	13	4th ,,	20th ,,	16	24th ,,	8th ,,	15
14th ,,	11th ,,	28	4th ,,	24th ,,	48	4th ,,	2nd Apl.	29	1st ,,	21st ,,	20	9th ,,	28th ,,	19	4th ,,	18th ,,	14				8th ,,	22nd ,,	14				24th ,,	15th ,,	22
23rd ,,	11th Mar.	47	6th ,,	30th ,,	52	4th ,,	8th ,,	35	1st ,,	21st ,,	20	28th ,,	8th June	11	4th ,,	15th ,,	11				10th ,,	22nd ,,	12						
27th ,,	25th Feb.	29	10th ,,	1st Apl.	50	7th ,,	11th ,,	35	2nd ,,	18th ,,	16	29th ,,	11th ,,	13	4th ,,	18th ,,	14				10th ,,	22nd ,,	12						
28th ,,	25th Mar.	56	11th ,,	30th Mar.	45	7th ,,	8th ,,	32	3rd ,,	21st ,,	18	29th ,,	11th ,,	13	5th ,,	19th ,,	14				15th ,,	28th ,,	13						
30th ,,	27th ,,	56	12th ,,	28th ,,	44	11th ,,	10th ,,	30	4th ,,	24th ,,	20	29th ,,	11th ,,	13	5th ,,	19th ,,	14				26th ,,	8th Sep.	13						
30th ,,	25th ,,	54	14th ,,	5th Apl.	50	11th ,,	6th ,,	26	4th ,,	25th ,,	22	29th ,,	11th ,,	13	5th ,,	17th ,,	12												
			16th ,,	1st ,,	44	15th ,,	15th ,,	31	4th ,,	20th ,,	16	30th ,,	13th ,,	14	5th ,,	17th ,,	12												
			18th ,,	28th Mar.	38	15th ,,	11th ,,	37	5th ,,	22nd ,,	17	30th ,,	15th ,,	16	5th ,,	17th ,,	12												
			19th ,,	2nd Apl.	41	16th ,,	13th ,,	28	6th ,,	22nd ,,	16	30th ,,	13th ,,	14	5th ,,	17th ,,	12												
			21st ,,	29th Mar.	35	17th ,,	20th ,,	34	6th ,,	25th ,,	19	30th ,,	14th ,,	15	5th ,,	17th ,,	12												
			21st ,,	2nd Apl.	40	18th ,,	16th ,,	29	6th ,,	25th ,,	19				5th ,,	19th ,,	14												
			26th ,,	2nd ,,	35	21st ,,	14th ,,	24	6th ,,	30th ,,	24				6th ,,	16th ,,	10												
			28th ,,	1st ,,	32	23rd ,,	18th ,,	26	7th ,,	23rd ,,	16				9th ,,	23rd ,,	14												
			28th ,,	3rd ,,	34	23rd ,,	14th ,,	22	10th ,,	22nd ,,	12				9th ,,	23rd ,,	14												
						24th ,,	22nd ,,	29	10th ,,	5th May	25				12th ,,	26th ,,	14												
						24th ,,	16th ,,	23	13th ,,	1st ,,	18				25th ,,	8th July	13												
						24th ,,	15th ,,	22	16th ,,	11th ,,	25				26th ,,	6th ,,	10												
						25th ,,	14th ,,	20	28th ,,	12th ,,	14				28th ,,	12th ,,	14												
						26th ,,	17th ,,	22																						
						26th ,,	17th ,,	22																						
						28th ,,	12th ,,	15																						
						28th ,,	1st May	34																						
						29th ,,	19th Apl.	21																						
						29th ,,	18th ,,	20																						
						31st ,,	19th ,,	19																						
						31st ,,	18th ,,	18																						
						31st ,,	19th ,,	19																						

TABLE XVIII

Insectary room temperature °C. (1931)

Period		Maximum temperature			Minimum temperature		
From	To	Highest	Lowest	Average	Highest	Lowest	Average
14th Jan. .	11th Feb. .	16·1	11·6	14·08	15·5	10·0	13·04
11th Feb. .	17th Mar. .	21·6	13·5	16·55	20·0	10·5	14·04
31st Mar. .	18th Apr. .	30·0	28·0	29·95	27·0	25·0	25·86
18th Apr. .	14th May .	35·0	29·0	32·57	30·0	26·0	27·67
6th June .	16th July .	37·0	27·0	33·63	34·4	24·4	30·02
16th July .	14th Aug. .	36·6	28·5	30·86	35·5	27·0	29·11
6th Aug. .	18th „ .	36·1	33·3	34·13	35·5	32·7	31·80
18th „ .	17th Sep. .	35·5	28·5	31·22	34·4	28·0	30·65
10th Sep. .	2nd Nov. .	26·3	22·4	23·28	24·1	21·4	22·30
2nd Nov. .	11th Dec. .	23·0	18·1	20·47	21·9	14·1	19·35

From January to the first week of March these cages were placed in the sun during the day and in the laboratory at night. Thus the egg-pods even laid at the same time were exposed to different temperatures. When the cages were placed in the sun, the eggs laid nearer the periphery of the cages received a greater quantity of heat; again the side of the cage facing the sun got hotter quicker. Perhaps all the egg-pods were not at the optimum soil moisture. For these reasons we find considerable variation in the incubation period of eggs laid at about the same time. Similar circumstances perhaps explain variations in Ballard's [1932] experiments. The average figures of each month are, however, quite significant.

In January-February the average incubation period was forty-three days in March 25·6 days, in April 18·7 days, from May to August eleven to fourteen days, in September sixteen days and in October 17·3 days. There was no oviposition in November and December.

During the summer months (May to August) the incubation period was eleven to fourteen days, which agrees with the incubation period at constant temperatures between 33° and 40°C.

It may be mentioned here that at Lyallpur, during January and February, oviposition and hatching would not have taken place if the cages had not been placed in the sun during day time. In all cases where the cages were left at the room temperature, no oviposition took place.

As already mentioned, Ballard and others [1932], working in Egypt, record the following incubation periods (days):—

November	106
December	95
January	79
February	67

It would appear that the soil temperature in these cages was below the developmental zero, most of the time. We do not agree with Ballard and his co-workers that the extended incubation period, which they obtained in some of their cages, during November-December, represented the winter diapause. We use the term 'diapause' in its true sense of a fixed rhythm [Uvarov, 1928]. It is interesting to note that in Ballard's experiments the eggs laid on November 6th, 1929, hatched after an incubation period of just nineteen days, while for those laid on November 24th, 1929, *i.e.*, eighteen days later, the incubation period was 116 days. As happened in our cages, Ballard's cages were also probably exposed to very different conditions of temperature and possibly humidity.

7. Duration of larval period

The duration of the larval period studied by different investigators in different countries has been given by Bodenheimer [1920] as follows: (To these we have added Ballard's figures):—

Author	Country	Larval duration (days)
Vosseler	East African Steppes	50
Do.	East African Mountains	60—70
King	Sudan	40—50
L'ellement	Algeria	44—53
Regiering	Morocoo	40
Gough	Egypt	42—56
Bucher	Palestine	42
Regnier	....	37—40
Bodenheimer	Palestine	42
Ballard and others	Egypt	39—153

From this it appears that the minimum larval duration, so far observed, is thirty-seven days and maximum 153 days. During the course of our study we have seen that the duration of the hopper stages varies with the quality and quantity of food, temperature, humidity, etc. It is, therefore, quite conceivable that wide variations may occur in cage experiments. Numerous observations in the field show that under natural conditions the two broods have very different larval duration; the summer brood taking about three to four weeks only and the spring brood (progeny of over-wintered adults) requiring six to seven weeks. This evidently directly depends on temperature.

I. Duration of the larval stages at constant temperatures

Three sets of experiments were performed in which hoppers were reared at the following constant temperatures: 24°, 27°, 30°, 33°, 36°, and 40°C. The conditions of the experiment may be briefly described thus: The cages employed were cylinders, made of brass wire-gauze (100 meshes to an inch), six inches long and two inches in diameter. These cylinders were closed at one end, and at the other end they were provided with a split cork through which the leaf of the food-plant could be introduced into the cage. In all these experiments young cabbage plants growing in earthen pots served as food. The cages were placed over living plants in pots, and the pots with the cages kept in thermostats at the required temperature. The hoppers had, therefore, an abundant supply of food available. The stage and conditions of hoppers were recorded at 8 A.M. daily. The data regarding the duration of the various stages at different temperatures, and mortality are given in Table XIX, and summarized in Table XX. At most temperatures four different cages were under observation and the number of hoppers reared at each temperature was not less than thirteen and in some cages the number was nineteen. The uniformity in the duration of a particular stage at each temperature is very significant.

TABLE XIX

History of hoppers reared at constant temperatures

(Experiment I. Reference No. L 224.)

Date	Days	24°C.	24°C.	27°C.	30°C.	30°C.	33°C.	33°C.	36°C.	36°C.	36°C.	40°C.
11th Nov. 1932 .	0	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)
12th „ .	1											
13th „ .	2											
14th „ .	3									II (3)		II (3)
15th „ .	4						II (3)	II (3)	II (3)		II (3)	
16th „ .	5				II (3)	II (3)						D (1)
17th „ .	6			II (3)								III (2)
18th „ .	7				D (1)				III (3)	III (3)	III (2)	
19th „ .	8	II (3)					III (3)	III (3)				
20th „ .	9		II (3)									
21st „ .	10		D (1)		III (2)	III (3)						IV (2)
22nd „ .	11			D (1)					IV (3)	IV (3)	IV (3)	
23rd „ .	12			III (2)			IV (3)	IV (3)				
24th „ .	13											
25th „ .	14					D (1)					D (1)	V (2)
26th „ .	15				IV (2)	IV (2)			V (3)	V (3)	V (2)	
27th „ .	16	III (3)	III (2)							D (1)		
28th „ .	17						V (3)	V (3)				
29th „ .	18											
30th „ .	19			IV (2)								
21st Dec. 1932	20				V (2)	V (2)						
2nd „ .	21											A (2)

23rd Dec. 1932 .	22								A (3)	A (2)	A (2)	
4th „ .	23											
5th „ .	24							D (1)				
6th „ .	25											
7th „ .	26	IV (3)					A (3)	A (2)				
8th „ .	27		IV (2)	D (1)								
9th „ .	28			V (1)								
10th „ .	29											
11th „ .	30											
12th „ .	31				A (2)	A (2)						
13th „ .	32											
14th „ .	33											
15th „ .	34											
16th „ .	35											
17th „ .	36											
18th „ .	37											
19th „ .	38											
20th ,	39	V (3)										
21st „ .	40		V (2)									
22nd „ .	41											
23rd „ .	42											
24th „ .	43											
25th „ .	44											
26th „ .	45											
27th „ .	46											
28th „ .	47			A (1)								
29th „ .	48											
30th „ .	49											
31st „ .	50											
1st Jan. 1933 .	51											

TABLE XIX—*contd.*

Date	Days	24°C.	24°C.	27°C.	30°C.	30°C.	33°C.	33°C.	36°C.	36°C.	36°C.	40°C.
2nd Jan. 1933. .	52											
3rd „ . .	53	D (1)										
4th „ . .	54											
5th „ . .	55											
6th „ . .	56											
7th „ . .	57											
8th „ . .	58											
9th „ . .	59	D (1)										
10th „ . .	60											
11th „ . .	61											
12th „ . .	62	A (1)	A (2)									
Duration of hopper stages in days.	...	62	62	47	31	31	26	26	22	22	22	21

NOTE.—Roman numbers represent instars of the hoppers.
Arabic numbers in ( ) represent the number of hoppers in the cage.
D-Died, and figure in ( ) represents number dead.
A-Adult, and figure in ( ) represents their number.

TABLE XIX—*contd.*

History of hoppers at different constant temperatures

(Experiment II. Reference No. P74)

Date	Days	24°C.	27°C.	30°C.	33°C.	36°C.	40°C.
14th Jan. 1933 . .	0	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)
15th ,, . .	1						
16th ,, . .	2	D (1)					
17th ,, . .	3			D (1)		II (3)	II (3)
18th ,, . .	4			II (2)	II (3)		
19th ,, . .	5						
20th ,, . .	6		II (3)			III (3)	III (3)
21st ,, . .	7						
22nd ,, . .	8	II (2)			III (3)		
23rd ,, . .	9			III (2)			D (1)
24th ,, . .	10					IV (3)	IV (2)
25th ,, . .	11						
26th ,, . .	12		III (3)		IV (3)		
27th ,, . .	13						
28th ,, . .	14			IV (2)		V (3)	V (2)
29th ,, . .	15						
30th ,, . .	16	III (2)					
31st ,, . .	17				V (3)		
1st Feb. 1933 . .	18		D (1)				
2nd ,, . .	19		IV (2)				
3rd ,, . .	20			V (2)			

TABLE XIX—*contd.*

Date	Days	24°C.	27°C.	30°C.	33°C.	36°C.	40°C.
4th Feb. 1933 .	21					A (3)	A (2)
5th ,, . .	22						
6th ,, . .	23						
7th ,, . .	24						
8th ,, . .	25						
9th ,, . .	26				A (3)		
10th ,, . .	27	IV (2)	V (2)				
11th ,, . .	28						
12th ,, . .	29						
13th ,, . .	30						
14th ,, . .	31			A (2)			
15th ,, . .	32						
16th ,, . .	33						
17th ,, . .	34						
18th ,, . .	35						
19th ,, . .	36						
20th ,, . .	37						
21st ,, . .	38						
22nd ,, . .	39	D (1)					
23rd ,, . .	40	V (1)					
24th ,, . .	41						
25th ,, . .	42						
26th ,, . .	43						
27th ,, . .	44						

TABLE XIX —*contd.*

Date	Days	24°C.	27°C.	30°C.	33°C.	36°C.	40°C.
28th Feb 1933 . .	45						
1st Mar. 1933 . .	46		A (2)				
2nd „ . .	47						
3rd „ . .	48						
4th „ . .	49						
5th „ . .	50						
6th „ . .	51						
7th „ . .	52						
8th „ . .	53						
9th „ . .	54						
10th „ . .	55						
11th „ . .	56						
12th „ . .	57						
13th „ . .	58						
14th „ . .	59						
15th „ . .	60						
16th „ . .	61						
17th „ . .	62						
18th „ . .	63	A (1)					
Duration of hopper stages (days).	..	63	46	31	26	21	21

TABLE XIX—*contd.*

History of hoppers at different constant temperatures

(Experiment III. Reference No L 276)

Date	Days	24°C.	27°C.	30°C.	33°C.	36°C.	40°C.
16th Jan. 1933 . .	0	I (7)	I (7)	I (7)	I (7)	I (7)	I (7)
17th ,, . .	1	D (1)	D (2)				D (1)
18th ,, . .	2						
19th ,, . .	3					II (7)	II (6)
20th ,, . .	4				II (7)		
21st ,, . .	5			II (7)			
22nd ,, . .	6		II (5)			III (7)	III (6)
23rd ,, . .	7						
24th ,, . .	8	II (6)			D (2)		
25th ,, . .	9	D (2)		III (7)	III (5)		
26th ,, . .	10					IV (7)	IV (6)
27th ,, . .	11						
28th ,, . .	12		D (1)			D (1)	
29th ,, . .	13		III (4)		IV (5)	D (1)	D (1)
30th ,, . .	14			IV (7)		V (5)	V (5)
31st ,, . .	15						
1st Feb. 1933 . .	16						
2nd ,, . .	17	III (4)					
3rd ,, . .	18				V (5)		
4th ,, . .	19						D (1)
5th ,, . .	20		IV (4)	V (7)			
6th ,, . .	21					A (5)	A (4)

TABLE XIX—*contd.*

Date	Days	24°C.	27°C.	30°C.	33°C.	36°C.	40°C.
7th Feb. 1933 . .	22						
8th ,, . .	23						
9th ,, . .	24			D (1)			
10th ,, . .	25						
11th ,, . .	26						
12th ,, . .	27	D (1)			A (5)		
13th ,, . .	28	IV (3)					
14th ,, . .	29		V (4)				
15th ,, . .	30						
16th ,, . .	31			A (6)			
17th ,, . .	32						
18th ,, . .	33						
19th ,, . .	34						
20th ,, . .	35						
21st ,, . .	36						
22nd ,, . .	37						
23rd ,, . .	38		D (1)				
24th ,, . .	39						
25th ,, . .	40						
26th ,, . .	41	V (2)					
27th ,, . .	42						
28th ,, . .	43						
1st Mar. 1933 . .	44						
2nd ,, . .	45						
3rd ,, . .	46						

TABLE XIX—*contd.*

Date	Days	24°C.	27°C.	30°C.	33°C.	36°C.	40°C.
4th Mar. 1933 . .	47		A (3)				
5th ,, . .	48						
6th ,, . .	49						
7th ,, . .	50						
8th ,, . .	51						
9th ,, . .	52						
10th ,, . .	53						
11th ,, . .	54						
12th ,, . .	55						
13th ,, . .	56						
14th ,, . .	57						
15th ,, . .	58						
16th ,, . .	59						
17th ,, . .	60						
18th ,, . .	61						
19th ,, . .	62						
20th ,, . .	63						
21st ,, . .	64	A (2)					
Duration of hopper stages in days.	..	64	47	31	27	21	21

TABLE XX

Summary of experiments set out in Table XIX

Temperature (°C.)	Duration of each instar (days) I	II	III	IV	V	Total duration in hopper stage (days)	Serial No. of experiments	No. of hoppers in each experiment	No. reaching the adult stage	Mortality (Per cent)
24 . .	8-9	7-9	10-11	13	21-23	62-64	1 2 3 4	3 3 3 7	1 2 1 2	63
27 . .	6	6-7	7	8-9	18-19	46-47	1 2 3	3 3 7	2 1 3	54
30 . .	4-5	4-5	4-5	5-6	11	31	1 2 3 4	3 3 3 7	2 2 2 6	25
33 . .	4	4-5	4	5	9	26-27	1 2 3 4	3 3 3 7	3 2 3 5	19
36 . .	3-4	3-4	4	4	7	21-22	1 2 3 4 5	3 3 3 3 7	3 2 2 2 5	37
40 . .	3	3	4	4	7	21	1 2 3	3 3 7	2 2 4	38

A second series of experiments was arranged in which hoppers were reared in glass jars covered with muslin, and were examined four times during the day, *i.e.*, at 7.0, 12.0, 16.0 and 21.0 hours, when food was changed, thereby securing a fairly high degree of accuracy regarding the exact time of moulting. In Table XXI the period under each stage is given as the minimum period of the stage *plus* half of the doubtful period.

TABLE XXI

Duration of larval instars at constant temperatures (1932)

Temperature (°C.)	Ref. No.	Date of hatching	I stage period	II stage period	III stage period	IV stage period	V stage period	Total larval life
			hrs.	hrs.	hrs.	hrs.	hrs.	Days hrs.
25	L 12	12th Jan. .	163·5					
	L 33	17th Jan. .						
			151·5					
			151·5	D				
			168					
	L 16	29th Jan. .	187	192	232·5			
			187	208·5	216			
			203·5	216	..			
			211		..			
			218·5		..			
	L 44	2nd Feb. .	156	192	192	292·5	487·5	56 ..
			160	192	192	292·5	..	
			160	192	192	..	..	
			180	172·5	..	..	..	
27	L 12	12th Jan. .	120	127·5	136·5	208·5	499·5	45 9
			136·5	127·5	156	220·5	..	
	L 34	15th Jan. .	101	..	..	..	..	

TABLE XXI—*contd.*

Temperature (°C.)	Ref. No.	Date of hatching	I stage period	II stage period	III stage period	IV stage period	V stage period	Total larval life
			hrs.	hrs.	hrs.	hrs.	hrs.	Days hrs.
	L 44	2nd Feb. .	124·5	132	..	..	..	
			129	144	..	..	..	
			139	144	..	..	..	
30	L 12	12th Jan. .	96	120	112·5	144	259·5	30 9
			96	151·5	96	136·5	256·5	30 14
		17th Jan. .	96	107·5	96	144	271·5	30 0
33	L 33	17th Jan. .	96	72	64·5	115	187	22 5
			96	72	72	120	247	24 5
			96	79·5	88·5	127	..	
			96	79·5	105	..	..	
	L 33	17th Jan. .	84	91·5	100	100	183	23 5
			96	88·5	91·5	100	192	23 14
	L 21	22nd Jan.	96	96	108	108	..	
36	L 12	12th Jan. .	74·5	64·5	96	84	156	20 0
			74·5	64·5	96	84	168	20 9
			74·5	81	79·5	84	172·5	20 14
	L 21	22nd Jan. .	79	..	..	..	..	.. ..
	L 44	2nd Feb. .	76·5	60	84	72	172	19 14
			76·5	67·5	76·5	76·5	203	21 6
			76·5	67·5	81	91·5	..	.. ..
			76·5	76·5	96	..	..	.. ..
37	L 33	17th Jan. .	60	67·5	88·5	79·5	172·5	19 9
			64·5	67·5	84	88·5	168	19 14

TABLE XXI—*contd.*

Temperature (°C.)	Ref. No.	Date of hatching	I stage period	II stage period	III stage period	IV stage period	V stage period	Total larval life
			hrs.	hrs.	hrs.	hrs.	hrs.	Days hrs.
			64·5	67·5	91·5	81	192	20 14
			64·5	72	87	81	216	21 14
			..	79·5	88·5	91·5	..	.. ..
	L 16	26th Jan. .	..	..	..	..	..	20 0
	L 56	13th Feb. .	83·5	67·5	76·5	96	168	20 14
			83·5	67·5	76·5	96	175·5	21 0
40	L 12	12th Jan. .	74·5	64·5	103	72	184·5	21 0
			74·5	81·5	87	81	..	.. ..
	L 33	17th Jan. .	67	64·5	67·5	100	151·5	19 0
			67	64·5	72	103	151·5	19 5
			67	64·5	77·5	108	172·5	20 14
	L 12	19th Jan. .	67	72	84	84	204	21 7
			67	79·5	81	103	..	.. ..
	L 44	2nd Feb. .	64·5	87	76·5	84	184	20 14
			64·5	87	76·5	..	..	.. ..
			64·5	91·5	72	132	..	.. ..
			64·5	91·5	76·5	..	..	.. ..

The results obtained may be summarised as follows:—

20°C. and below.—Hoppers could not complete their development below 20°C. Freshly hatched hoppers lived as long as thirty-one days at 20°C., and died without moulting. They ate very little food and the original body colour (green or black) darkened very much. It is proposed to investigate the influence of this range of temperature on the hoppers of different stages.

21°-25°C.—Development took place very slowly and the death rate was enormous. At 21°C., the hoppers took thirteen to sixteen days in each of the first and second stages, but could not be bred further.

Out of 124 hoppers kept for breeding, in twenty-one batches, at this range of temperature, only seven acquired wings, taking fifty-six days at 25°C., and sixty-one to sixty-three days at 24°C. during the larval period.

At 27°C., the larval duration was forty-five to forty-seven days.

At 30°C., the average duration of the total larval life was thirty to thirty-one days. Bodenheimer [1930] also bred freshly hatched larvae in a thermostat at 30°C., but the results obtained by him do not agree with ours and for facility of comparison we give them below:—

	I instar	II instar	III instar	IV instar	V instar	Total larval period
	days	days	days	days	days	days
Bodenheimer's.	7	13	7	6	10	43
At Lyallpur .	4-5	4-5	4-5	5-6	11	31

At 33°C., the larval duration was twenty-two to twenty-seven days; and at 36°C., twenty to twenty-one days. Further rise in temperature did not shorten the larval duration, so that at 37°C. and 40°C., it was almost the same as at 36°C. This represents the range of temperature at which *Schistocerca gregaria* has the minimum larval duration of nineteen to twenty-one days.

44°C.—When the temperature was raised to 44°C., the larval duration was distinctly prolonged and the death rate increased enormously. Out of forty-four hoppers kept for breeding, in twelve different batches, at this temperature, only one acquired wings in twenty-seven days. Incidentally it may be mentioned that at this temperature the hoppers ate very little, appeared excited and lost their black markings.

For the calculation of the thermal constant we have the following data:—

Duration of the hopper stage at 25°C. . =56 days.
Duration of the hopper stage at 36°C. . =20 days.
Therefore (25-x) 56 . . . . = (36-x) 20.
x (Developmental zero) . = 18·9°C.
Thermal constant . . . . . = 342.

In the following table (Table XXII) we compare the calculated figures with the empirical ones.

TABLE XXII

The empirical and calculated figures for larval duration of Schistocerca gregaria.

Temperature (°C.)	Empirical duration (days)	Calculated larval duration (days)	Deviation of calculated figures from the empirical (Per cent)
25	56	56	0·0
27	42·2	45·3	7·3
30	30·8	30·2	—1·9
33	24·2	24·3	0·4
36	20	20·4	2·0

When one takes into consideration the fact that besides temperature there are other factors which influence duration in the hopper stage, such as food—quality and quantity—and humidity, etc., one is forced to admit that the approach of the empirical figures to the calculated figures is very significant.

The time-temperature and temperature-rate curves for the larval duration are shown in Fig. 6. The points expressing the index of development fit very closely on a straight line. The theoretical threshold of development lies between 18 and 19°C.

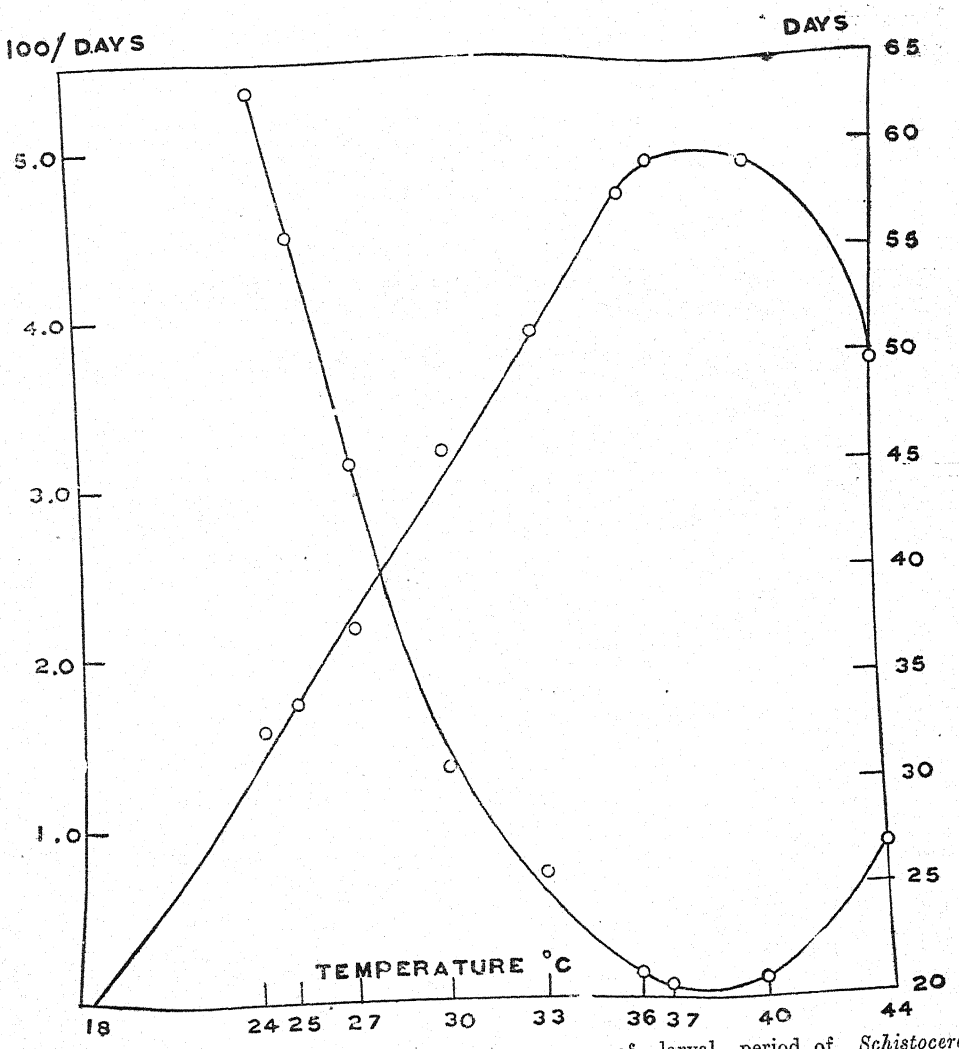

Fig. 6. Time-temperature and temperature-rate curves of larval period of *Schistocerca gregaria.*

II. Upper vital limit.

A number of observations were carried out by rearing hoppers at 46°C. As it was impossible for hoppers of any stage to moult at this temperature, freshly hatched and freshly moulted hoppers in various stages were exposed singly in each jar. Food was changed five times every day—at seven hours in the morning, three times during the day, and at twenty-one hours at night. Of the twenty-one hoppers under observation not a single one moulted. Table XXIII gives the details of these experiments. At this temperature the hoppers of the first stage lived as long as eight days and nineteen hours, those of II stage for four days, and those of III and V stages for six days and sixteen

hours without moulting. The hoppers at this temperature are too much excited and hardly take any food.

TABLE XXIII

Duration of life of hoppers at 46°C.

(In each jar a single hopper was kept.)

Stage	No. of observations	Experiment started		Died		Period	
		Date (1932)	Time	Date (1932)	Time	Days	Hrs.
I (Freshly hatched)	1	29th Feb.	16·0	2nd Mar.	7·0	1	15
	2	29th ,,	16·0	6th ,,	13·0	5	21
	3	1st Mar.	10·0	4th ,,	7·0	2	21
	4	6th ,,	12·0	15th ,,	7·0	*8*	*19**
II (Freshly moulted).	1	4th ,,	12·0	6th ,,	13·0	2	1
	2	4th ,,	12·0	6th ,,	13·0	2	1
	3	8th ,,	15·0	9th ,,	16·0	1	1
	4	15th ,,	12·0	17th ,,	7·0	1	19
	5	17th ,,	10·0	21st ,,	10·0	*4*	*0*
III (Freshly moulted).	1	4th ,,	12·0	5th ,,	16·0	1	4
	2	4th ,,	12·0	5th ,,	16·0	1	4
	3	8th ,,	15·0	15th ,,	7·0	*6*	*16*
V (Freshly moulted).	1	4th ,,	12·0	5th ,,	7·0	0	19
	2	4th ,,	12·0	5th ,,	7·0	0	19
	3	4th ,,	12·0	5th ,,	..	0	21
	4	4th ,,	12·0	5th ,,	..	0	19
	5	8th ,,	15·0	9th ,,	12·0	0	21
	6	15th ,,	12·0	16th ,,	7·0	0	19
V (Freshly moulted).	1	4th ,,	12·0	5th ,,	21·0	1	9
	2	8th ,,	15·0	15th ,,	7·0	*6*	*16*
	3	15th ,,	12·0	16th ,,	7·0	0	19
Adult (male) .	1	10th ,,	11·0	12th ,,	7·0	1	20

NOTE.—None of the hoppers in these experiments moulted.

* Figures in *italics* indicate the longest duration of life.

Thus at 46°C., the larvae of *Schistocerca gregaria* are not able to develop and moult. At optimum temperature the hoppers of the first four stages moult to the next stage within three to four days while at 46°C. they lived for more than this period without moulting.

Thus we find that 46°C. represents the upper vital limit of development for the larvae of *Schistocerca gregaria*.

III. Duration of the hopper stages at laboratory temperature

The duration of each instar and the total hopper period at room temperature for every fortnight from March to October, are given in Table XXIV, and the room temperature record in Table XXV. The total hopper period is expressed in two columns:—(1) average duration of hopper stages, *i.e.*, when half of the hoppers of a batch acquired wings, and (2) shortest duration, *i.e.*, the time taken by the 1st individual of the batch to acquire wings.

Table XXIV

Duration of larval instars (days) for hoppers hatched during different months (1931) and bred at room temperature

Period of hatching (Date and month) 1932	I stage	II stage	III stage	IV stage	V stage	Total larval period	
						Average	Shortest
19th—29th Mar. . .	7·2	4·4	6·4	9·6	10·7	39·8	34·8
1st—15th April . .	7·5	6	6·1	6	9·8	35·7	33
16th—30th ,, . .	5·2	5	5·7	7	9	31·5	30·1
2nd—11th May . .	4·8	4·8	6·4	5·8	9	32·2	31·2
19th—31st ,, . .	4·4	4·7	6·2	7·1	8·7	31·5	31
1st—17th June . .	4·7	4·6	5·7	5·7	8·6	29·5	27·7
6th—15th July . .	4·6	4·7	6·1	6	8·1	29·3	27·8
18th—28th ,, . .	5·3	5·2	5·5	5·2	8	29·7	29·2
10th—29th Aug. . .	5·5	6·5	6·6	6·8	11·2	36·7	32·3
3rd—30th Sept. . .	6	6·2	6·5	6·5	10·2	35·5	32·7
23rd—31st Oct. . .	18·6	33					
		One hopper moulted and entered III instar: rest died in II stage after 30-44 days.					
3rd—13th Nov. . .	20·2	Died after living for 19-21 days in II stage.					
17th—20th ,, . .	25	One hopper moulted and entered II instar. rest died in I instar after living for 36 days.					

TABLE XXV

Record of room temperature °C. (1931).

Period	Maximum temperature		Minimum temperature	
	Highest	Lowest	Highest	Lowest
March	29·0	15·5	27·0	14·4
April	33·0	28·0	30·0	28·0
May	35·5	25·0	31·0	22·5
June	37·2	30·5	33·7	27·7
July	37·7	27·7	34·4	24·4
August	36·1	28·0	35·5	28·0
September	33·5	28·5	31·6	28·0
October	31·3	23·5	31·9	21·6

It will be observed that the individual instars as well as the total duration vary with temperature. During March the larval duration was forty days. As the temperature rose the duration shortened, the minimum duration recorded at laboratory temperature being twenty-four days for the hoppers hatched on 10th July 1932. During this period the temperatures recorded were:—

Highest maximum 35·5°C. Highest minimum 32°C.

Lowest maximum 33°C. Lowest minimum 28·5°C.

The larval duration again began to increase in August reaching thirty-eight days in September, Hoppers hatched in October and kept at room temperature could not complete their life cycle. They took sixteen to twenty days in I stage, and twenty-one to thirty-three days in II stage, and the majority of them died before the 2nd moult.

The hoppers hatching early in November were able to pass through only the 1st moult taking, on an average, twenty days in I stage. All of them died before the 2nd moult, living for another tewenty days in II stage.

The hoppers hatching at the end of November could not moult even once and died after living for thirty-six days in I stage.

IV. Duration of larval stages in the hoppers caged on plants in the open during winter (February—March)

A number of experiments were started by rearing hoppers in muslin cages on living plants growing in the fields. Only in one case did the hoppers exposed to these conditions reach the adult stage. Details of the observations are as follows:—

The hoppers which had hatched on 12th October, 1932 were caged on a cotton plant in a muslin cage. They were transferred to a cabbage plant on 27th December. These hoppers remained at suitable temperature during the warmer part of the day only, and throughout the night and during the greater portion of the day the temperature remained below the development zero. This was particularly so during the III and IV stages. These two hoppers acquired wings on 22nd March, 1933, *i.e.*, after 161 days. This interesting observation shows the influence of temperature in prolonging the hopper stages and the possibility of the locust passing winter in the nymphal stages.

Ballard and co-workers have recorded a similar case and it is a happy coincidence that the period of their experiment synchronises with that of ours, and further the temperatures at which the two experiments were conducted were more or less the same (Table XXVI). It is, however, necessary to make it quite clear that both in our experiments and in Ballard's observations, the temperatures given are not the temperatures inside the cages but are taken from the meteorological records of the stations. In both cases the mean maximum and mean minimum temperatures for the different months are given, and evidently on certain days the temperature was higher than the mean maximum. These two experiments carried out in two different countries, during two different years, giving almost identical results are very significant and prove the great influence of temperature on the duration of the life of the Desert Locust hoppers.

TABLE XXVI

Comparison of larval duration at Lyallpur and in Egypt

1. Lyallpur. Reference No. P 30.

Hatching	1st moult	I stage (days)	2nd moult	II stage (days)	3rd moult	III stage (days)	4th moult	IV stage (days)	5th moult	V stage (days)	Total nymphal life (days)
12th Oct. 1932 .	24th Oct.	12	6th Nov.	13	16th Dec.	40	26th Feb. 1933.	7 :	22nd Mar.	24	161
		Oct.	Nov.		Dec.	Jan.	Feb.		March.		
Temperature °C.	Mean max..	32·7	28·6		20·8	17·4	22·8		27·9		
	Mean min.	14·6	6·4		4·9	2·3	6·9		11·5		

2. Ballard (Egypt). Reference No. Cage No. 11.

16th Oct. 1928 .	28th Oct.	12	10th Nov.	13	23rd Nov.	13	16th Jan. 1929.	54	18th Mar.	61	153
		Oct.	Nov.		Dec.	Jan.	Feb.		March.		
Temperature °C.	Mean max.	28·5	25·9		21·1	18·9	20·2		22·9		
	Mean min.	15·8	12·2		7·7	4·9	5·0		6·6		

8. Longevity of the winged stage

The life of adults (in days) from the date of acquiring wings to the date of death at four constant temperatures is given in Table XXVII. The average durations of all the observations at each temperature are given in Table XXVIII.

TABLE XXVII

Longevity of adults at different constant temperatures

Temperature (°C.)	Ref. No.	Date of acquiring wings		Date of death		Adult life	
		Male	Female	Male	Female	Male	Female
40	40 IA_1	29th Jan. 1932.	29th Jan. 1932.	1st Mar. 1932.	26th Feb. 1932.	32	28
				14th Apl. 1932.			
	40 IA_2	,,	,,	26th Feb. 1932.	29th Feb. 1932.	28	31
				29th Feb. 1932.	12th Mar. 1932.	31	43
	40 IA_3	,,	,,	2nd Mar. 1932.	28th Feb. 1932.	33	30
				10th Mar. 1932.		41	
	40 IIA_1	14th Mar. 1932.	14th Mar. 1932.	14th Apl. 1932.	22nd Apl. 1932.	27	39
	40 IIA_2	,,	,,	4th Apl. 1932.	11th Apl. 1932.	21	28
				13th Apl. 1932.	14th Apl. 1932.	30	31
					Average life (days).	35	33
36	C_1	8th Dec. 1931.	8th Dec. 1931.	13th Jan. 1932.	31st Jan. 1932.	36	54
					31st Jan. 1932.		54
	IV_1	15th Sept. 1931.	15th Sept. 1931.	...	22nd Oct. 1931.	...	37
	IV_2	15th Sept. 1931.	14th Sept. 1931.	10th Dec. 1931.	26th Oct. 1931.	87	41
	III_1	13th July 1931.	13th July 1931.	26th Aug. 1931.	11th Aug. 1931.	44	29
	III_2	,,	,,	,,	14th Aug. 1931.	...	32
	III_3	24th July 1931.	,,	30th Aug. 1931.	16th Aug. 1931.	37	34
				30th Aug. 1931.		37	
	T_2	14th May 1931.	14th May 1931.	29th June 1931.	7th June 1931.	46	24
				29th June 1931.	17th June 1931.	46	34
					30th June 1931.		47

TABLE XXVII—*contd.*

Longevity of adults at constant temperatures.

Temperature (°C.)	Ref. No.	Date of acquiring wings		Date of death		Adult life	
		Male	Female	Male	Female	Male	Female
	T_3 . .	18th May 1931.	18th May 1931.	11th June 1931.	25th June 1931.	24	38
				27th June 1931.	29th June 1931.	40	42
					30th June 1931.		43
	N_1 . .	10th Mar. 1931.	12th Mar. 1931.	14th Apl. 1931.	...	35	...
			13th Mar. 1931.		6th Apl. 1931.		35
					18th Apl. 1931.		36
	N_2 . .	17th Mar. 1931.	17th Mar. 1931.	20th Apl. 1931.	14th Apl. 1931.	34	28
	L_{60} . .	27th Jan. 1932.			21st Feb. 1932.	...	25
		24th Feb. 1932.	24th Feb. 1932.	9th Mar. 1932.	21st Mar. 1932.	14	26
					Average life (days).	39·7	36
30 . .	30 B . .	21st Jan. 1931.	10th Jan. 1931.	16th Mar. 1931.	23rd Mar. 1931.	54	72
	30 A . .	„	„	17th March. 1931.	17th Mar. 1931.	55	66
	29 C . .	10th Jan. 1931.	„	8th Mar. 1931.	17th Mar. 1931.	57	66
	29 B . .	„	„	23rd Mar. 1931.	11th Feb. 1931.	72	60
	29 A . .	„	„	26th Mar. 1931.	8th Mar. 1931.	75	57
					Average life (days).	62·6	64
27 . .	...	18th Dec. 1930	...	12th Mar. 1931.	...	85	...

TABLE XXVIII

Longevity of adults at constant temperatures

(Summary)

Temperature (°C.)	No. of observations	Average adult life (days)	
		Male	Female
40	16	35	33
36	30	39·7	36
30	10	62·6	64
27	1	..	85

It will be noticed that as the temperature falls from 40° C. to 27° C., the duration of adult life increases.

A number of observations on the duration of adult life at room temperature from April to November, 1930, are given in Table XXIX. The average durations of all observations in different months are given in Table XXX. Adults dying within a week of their acquiring wings have been excluded from the record presented in these tables, since such deaths, most definitely, must have occurred as the results of abnormal causes.

TABLE XXIX

Longevity of adults kept at room temperature.

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
N7 b3 .	Male	1st April 1931	18th May 1931.	47
	Female . . .	,,	22nd May 1931.	51
I_{22} . .	Males	4th Apl. 1931	17th June 1931.	74
			21st June 1931.	73
			13th July 1931.	100
	Female . . .	,,	2nd July 1931.	39
Nil .	Male	9th Apl. 1931	1st June 1931.	53
	Female . . .	5th Apl. 1931	19th May 1931.	44
I_9 . .	Males	18th April 1931.	28th May 1931.	40
			2nd June 1931.	45
I_3 . .	Male	28th April 1931.	30th May 1931.	32
	Female . . .	,,	15th June 1931.	48

TABLE XXIX—*contd.*

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
I_9 . .	Females . . .	29th April 1931.	8th June 1931.	40
			6th June 1931.	38
I_{14} . .	Male	30th April 1931.	30th May 1931.	30
			Average .	53·9
I_{11} . .	Male	2nd May 1931	23rd June 1931.	52
I_{10} . .	Male	5th May 1931	26th May 1931.	21
	Female . . .	,,	17th June 1931.	43
I_{13} . .	Male	,,	19th June 1931.	45
	Female . . .	,,	23rd June 1931.	49
I_{15} . .	Males	,,	9th June 1931	35
			1st July 1931.	57
I_{14} . .	Male	6th May 1931	9th July 1931	64
	Female . . .	,,	14th July 1931.	69
I_7 . .	Male	7th May 1931	21st June 1931.	45
	Female . . .	,,	15th June 1931.	39

TABLE XXIX—*contd.*

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
I_{12} . .	Male	8th May 1931	26th June 1931.	4
	Females . . .	,,	2nd June 1931.	2
			8th June 1931.	31
			9th June 1931.	32
			2nd Aug. 1931.	86
I_{11} . .	Male	10th May 1931.	18th June 1931.	39
R43d .	Male	16th May 1931.	29th June 1931.	44
	Female . . .	,,	26th June 1931.	41
II_6 . .	Male	16th May 1931.	27th June 1931.	42
	Female . . .	,,	3rd June 1931	18
I_{15} . .	Females . . .	20th May 1931.	30th May 1931.	10
		22nd May 1931.	8th July 1931.	47
			Average .	42·74
Q_2 . .	Male	15th June 1931	27th July 1931	42
	Female . . .	,,	8th Aug. 1931	54
Q_3 . .	Male	,,	20th July 1931	35
	Female . . .	,,	25th July 1931	40

TABLE XXIX—*contd.*

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
Q_4 . .	Male	15th June 1931	8th Aug. 1931	54
	Female . . .	,,	3rd Aug. 1931	49
Q14(i) .	Male	,,	8th July 1931	23
	Female . . .	,,	24th July 1931	39
Q14(ii) .	Male	,,	24th July 1931	39
	Female . . .	,,	21st July 1931	36
Q15(i) .	Female . . .	,,	15th July 1931	30
Q15(ii) .	Female . . .	,,	15th July 1931	30
II_1 .	Male	24th June 1931	17th Aug. 1931	54
	Female . . .	,,	18th Aug. 1931	55
II_2 .	Female . . .	,,	15th July 1931	21
II_3 .	Male	,,	23rd July 1931	29
II_4 .	Male	,,	6th July 1931	12
II_3 .	Male	26th June 1931	15th July 1931	20
	Female . . .	,,	22nd Sept. 1931	88
II_4 .	Males	26th to 29th June 1931	4th July 1931	5-8
	Females . . .	,,	4th Aug. 1931	36-39
II_5 . .	Males	26th June 1931	22nd Aug. 1931	57

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
II_3 .	Male	27th June 1931	15th July 1931	18
	Female . . .	,,	29th July 1931.	32
II_4 .	Male	,,	4th July 1931	7
	Female . . .	,,	4th Aug. 1931	38
II_5 . .	Male	,,	30th July 1931.	33
	Female . . .	,,	16th Aug. 1931	50
			Average .	35·9
II_4 .	Male	5th July 1931	29th July 1931	24
II_7 .	Male	12th July 1931	6th Sept. 1931	56
	Female . . .	,,	30th Aug. 1931	49
Q16 .	Male	13th July 1931	1st Sept. 1931	50
Q1 . .	Males	11th—15th July 1931	31st Aug. 1931	47-51
	Female . . .	11th July 1931	11th Sept. 1931	62
II_2 .	Males	22nd July 1931	1st Aug. 1931	10
			4th Sept. 1931	44
	Female . . .	,,	25th Aug. 1931	34
II_{11} .	Male	,,	25th Aug. 1931	34

TABLE XXIX—*contd.*

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
II_{13} .	Male	24th July 1931	30th Aug. 1931	37
	Female . . .	,,	11th Sept. 1931	49
II_{14} .	Male	,,	30th Aug. 1931	37
	Female . . .	,,	2nd Sept. 1931	40
II_{15} .	Male	,,	26th Aug. 1931	33
	Female . . .	,,	9th Sept. 1931	47
	Female . . .	,,	31st Aug. 1931	38
			Average .	40·61
II_{8} .	Male	2nd-3rd Aug. 1931	8th Aug. 1931	5-6
	Female . . .	,,	11th Sept. 1931	39-40
II_{17} .	Male	3rd-8th Aug. 1931	9th Sept. 1931	29-34
	Female . . .	2nd-5th Aug. 1931	6th Sept. 1931	32-35
II_{18} .	Male	22nd Aug. 1931	2nd Sept. 1931	11
	Female . . .	,,	27th Sept. 1931	36
			Average .	26·7
39a .	Female . . .	16th Aug. to 6th Sept. 1930	13th April 1931	219-240

TABLE XXIX—*contd.*

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
39b .	Male	16th Aug. to 6th Sept. 1930	19th April 1931	225-245
	Female . . .	,,	7th April 1931	213-234
			Average .	229·33
47a .	Male	2nd-7th Oct. 1930	25th April 1931	200-205
	Female . . .	,,	,,	200-205
47b .	Male	,,	30th April 1931	205-210
	Female . . .	,,	13th April 1931	188-193
48a .	Male	,,	26th April 1931	201-206
	Female . . .	,,	19th April 1931	194-199
35 . .	Male	17th Oct. 1930	18th April 1931	183
	Female . . .	,,	30th April 1931	195
8 . .	Male	18th Oct. 1930	4th May 1931	198
	Female . . .	,,	27th April 1931	192
13 . .	Male	,,	30th April 1931	195
	Female . . .	,,	2nd April 1931	167
18a .	Male	20th Oct. 1930.	3rd April 1931	166
	Female . . .	,,	10th April 1931	173
18b .	Female . .	,,	11th April 1931	174

TABLE XXIX—*contd.*

Cage No.	Sex	Date of acquiring wings	Date of death	Adult life (days)
49a .	Male	27th-31st Oct. 1930.	26th Feb. 1931	118-122
	Female . . .	,,	5th April 1931	156-160
50b .	Male	,,	8th April 1931	159-163
	Female . . .	,,	30th April 1931	181-185
51b .	Male	,,	17th April 1931	168-172
	Female . . .	,,	6th April 1931	157-161
53 . .	Male	,,	11th April 1931	162-166
	Female . . .	,,	6th April 1931	157-161
54 . .	Female . . .	,,	7th April 1931	158-162
			Average .	178·4
40a .	Male	16th Oct. to 24th Nov. 1930.	19th April 1931	147-186
	Female . . .	,,	12th April 1931	140-179
40b .	Male	,,	25th April 1931	153-192
	Female . . .	,,	12th April 1931	140-179
41 . .	Male	16th Oct. to 3rd Nov. 1930.	19th April 1931	168-186
	Female . . .	,,	17th April 1931	166-184
46b .	Male	,,	22nd April 1931	171-189
	Female . . .	,,	14th April 1931	163-181
			Average .	170·25

TABLE XXX

Average longevity of adults during different months at room temperature

Month in which wings acquired	No. of observations	Average adult life (days)
April	15	53·9
May	23	42·7
June	28	35·9
July	17	40·6
August	6	26·7
September	3	229·3
October	24	178·4
November	8	170·3

It will be observed that from April onwards as the season gets warmer the duration of the adult life decreases. It is minimum from June to August and begins to increase after this. Under laboratory conditions the tendency to over-winter begins to appear in adults acquiring wings in the end of August, while almost all the adults acquiring wings from September to November over-wintered.

9. Total life and number of broods in a year

As already mentioned, various views have been entertained regarding the number of broods of *Schistocerca gregaria* in a year, but all the investigators hold one thing in common, *i.e.*, *Schistocerca gregaria* undergoes a compulsory long diapause in the adult stage thus restricting its broods to only one or two per year. Vayssiere [1932], King [1922], Uvarov [1928] and Bodenheimer [1929] are of opinion that the Desert Locust has only one generation in the year with a long diapause in the adult stage. On the other hand Cotes [1891] Künckel [1905], Vosseler [1905] and Regnier, Lespes and Rungs [1931] are inclined to think that there are two generations in a year, the adults of the winter generation undergoing a long diapause. Ballard and others [1932] are of opinion that under favourable natural conditions there may be three broods in a year—a spring generation, a summer generation and an autumn generation.

Our observations definitely prove that there are normally two distinct broods in nature under Indian conditions. The over-wintered females begin laying eggs in February and die out by the end of May. In the second brood the oviposition starts in July and adults die off before November.

Experiments conducted at constant temperatures go to prove that there is no diapause in *Schistocerca gregaria* in any stage of its life-cycle and that the duration in various stages mainly depends upon external factors and particularly temperature. Thus, given suitable temperature (27°C. – 40°C.) throughout the year, *Schistocerca gregaria* may have more than two broods.

The average total life and the number of broods which may be reared in the laboratory at different constant temperatures are given in Table XXXI.

TABLE XXXI

Average total life (days) and number of broods of Schistocerca gregaria *at different constant temperatures*

Temperature (°C.)	Egg stage	Larval stage	Adult stage	Total life	Pre-oviposition period	Duration of one brood	No. of broods in a year
40 .	11·0	20·7	34	65·7	14·3	46·0	7·9
36 .	12·0	21·2	37·8	71·0	17·6	50·8	7·2
30 .	16·0	30·9	63·3	110·2	24·2	71·1	5·1
27 .	20·0	45·4	85	150·4	32·0	97·4	3·7

It will be observed that it is not the total life of the insect which has been taken into consideration for calculating the number of broods in a year, but the duration of the egg stage, the larval stage and the pre-oviposition period put together give the duration of one brood. The cycle, therefore, is from egg to egg. Once oviposition has occurred, the life of the adult has no control on the life-cycle and its duration.

The number of broods at laboratory temperature.—As already pointed out the earliest eggs laid in the last week of February take three to four weeks to hatch, the hopper stage lasts for five to six weeks and the pre-oviposition period in early summer requires another two to three weeks; so that the adults of the first brood are usually ready to lay eggs in the 2nd and 3rd weeks of June. The second brood falling in the hot months completes the life cycle quicker. The adults may appear in the beginning of August and oviposition may commence after another two weeks. The temperature now begins to fall and the larvae of 3rd brood reach adult stage from the middle of October to middle of November. These adults over-winter and produce next generation in early spring. Thus, under laboratory conditions three broods of *Schistocerca gregaria* can be bred in a year. (For laboratory temperature record see Tables XVIII and XXV).

10. Summary.

The biology of *Schistocerca gregaria* has been studied at constant temperatures and general conclusions confirmed by a large number of observations at room temperature.

Pre-copulation and pre-oviposition period or, in other words, the sexual maturity of adults depends on temperature. It varies from 14·2 days at 40°C. to thirty-two days at 27°C. If, however, a temperature below 20°C. prevails for a long time, this period may be very much prolonged. This is why under natural conditions the adults of the over-wintered brood take six to seven months to commence oviposition. Under laboratory conditions a maximum number of eleven packets of eggs was obtained, each being laid at an interval of three days to three weeks depending on temperature.

The theoretical threshold of development of eggs lies at 17°-18°C. The incubation period varies from eighty-five days at 18·5°C. to a minimum of nine days at a temperature above 40°C. Eggs exposed to 46°C. for eight hours or more do not develop.

The larvae of *Schistocerca gregaria* cannot complete their development at 20°C. and below. The larval period is sixty-two to sixty-three days at 27°C., while it is only twenty days at temperatures between 36° and 40°C. The larvae are unable to moult at 46°C., which, therefore, represents the upper vital limit for this species.

The longevity of the adults is inversely proportional to the environmental temperature. At temperatures above 36°C. the adult life is about one month. At temperatures below 30°C., the adults may live for two to three months or even more.

Under natural conditions in the Punjab there are only two broods in a year. The adults of the over-wintered brood lay eggs from February to April, and die out by May, those of the summer brood lay eggs from July to September. Under favourable conditions of temperature, however, the locust can breed quicker and produce several broods in a year. These observations definitely prove that there is no diapause in *Schistocerca gregaria* in any stage of its development and the duration of various stages depends mainly upon environmental conditions of which temperature is by far the most important.

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STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.*

III. WHY LOCUSTS EAT WOOL

A study in the hydromania of Schistocerca gregaria

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CONTENTS

	PAGE
I. INTRODUCTION	263
II. PROBLEM	264
III. EXPERIMENTS AND OBSERVATIONS	264
IV. DISCUSSION	266
V. SUMMARY	267
REFERENCES	267

I. INTRODUCTION

Locusts are notorious for their unappeasable hunger, and for a voracity rarely equalled elsewhere in the animal kingdom. Their dietary covers a very wide range of vegetation. There are exceedingly few cultivated plants that they would refuse to feed on and very few wild ones which they would not eat.† Nor do they confine their destructive activities to soft leaves and juicy green twigs. They are known to gnaw the hard bark of *babul* (*Acacia arabica*)

*Studies on *Schistocerca gregaria* Forsk.

I. The micropyle in *Schistocerca gregaria* Forsk. and some other Acridiidae. *Ind. J. Agric. Sci.* **3**: 639-645. 1933.

II. The biology of the desert locust with special relation to temperature. *Ind. J. Agric. Sci.* **6**: 188-262. 1936.

† It is implied that the locusts are absolutely indiscriminate feeders. There are a few plants which they would refuse to eat. There are others which are eaten in case of extreme privation only.

trees till the wood is laid bare and the tree looks white from root to top. In the diary of a *Naib* of Bhag (Kalat, Baluchistan) a case is recorded from village Audro Qadir Bux (May 1924) where "a large number of hoppers having hatched out attacked wheat that had been reaped, heaped and blown". This appears rather extraordinary and without verification one is not prepared to accept this statement, but there is 'no telling', so out of the ordinary are these 'beasts'! They are known to nibble at window and door frames, make a meal of thatched roofs of houses and similar other materials and eat up their own disabled and dead comrades.* An unfortunate individual undergoing a moult sometimes becomes an object of a concerted attack by its comrades. Hoppers while just emerging from the soil are sometimes feasted upon by others which had hatched out earlier from the same egg cluster [Nikolsky, 1925]. Unconfirmed reports of their attack on human bodies are also on record.

Nikolsky [1925] recorded cases where the hoppers of Asiatic Locust (*Locusta migratoria* L.) ate off huge holes through felt tents. On their march through a village they gnawed through curtains and window blinds of dwellings and attacked bags and luggage.

Faure [1932] states: "Where grass is scarce the Brown Locusts (*Locustana paradilana* Walker) will no doubt eat almost anything and one hears of clothings, curtains and the like having been damaged during recent invasions."

The most extraordinary, and perhaps amusing, instance of all perversions is recorded by Nikolsky [1925], in regard to a swarm of hoppers which had appeared among a flock of sheep at night and by the morning had caused the flock to assume a closely shorn appearance, except for small patches of wool round the eyes.

II. Problem

Why do locusts eat wool? Is it due to hunger? Or is it because of a 'hydromania'—an intense yearning for moisture?

Wool is a material which evidently can have practically no nutritive value for an insect, the digestive system of which is not adapted for such a diet. To discover the reason for this curious phenomenon of wool eating a series of experiments were conducted.

III. Experiments and observations

(i) In a preliminary set of experiments some raw wool†, previously dried and weighed, was soaked in water and placed in a cage, alongside a fresh cabbage leaf, the usual food provided for the locusts bred in the laboratory. Two pairs of locust fliers were kept in this cage. Fresh leaves were supplied four times

* The habit of eating their dead comrades is of practical importance. If the death be due to eating poison baits, the eater of dead body may itself die of poison. Granovsky [1926] has practically observed this.

† Raw wool was obtained fresh from local butchers.

a day. After forty-eight hours the wool was removed from the cage, dried and weighed again. It showed no difference in weight. The leaves provided were eaten in all cases. Observations made in six repetitions of this experiment pointed to the conclusion that the Desert Locust has, at least, no preference for wool and will not eat it when green food is available.

(ii) In another set of experiments, the fliers were kept in a desiccator in which low humidity was maintained by means of concentrated sulphuric acid. They were kept without food for twenty-four hours at a temperature of 37°C. At the end of this period some dry wool was introduced into this desiccator. The fliers came to it but rejected it after a casual 'inspection'. After an hour some more wool soaked in water was introduced. This was eagerly eaten though the quantity eaten was not large. As dry wool is not acceptable to the hungry and thirsty locusts it is apparent that moisture in the soaked wool is the only factor which determines its acceptance.

It is also interesting to note here that a microscopic examination of the fæces of the locusts that had fed on wool showed wool fibres, which evidently had suffered no physical change in passing through the alimentary tract, except that the wool fibres had been cut into pieces by the action of the mandibles.

(iii) In the third set of experiments the fliers were starved under similar conditions as above, but at the end of a period of twenty-four hours a green cabbage leaf and some soaked wool were simultaneously introduced into the desiccator. The fliers took the shortest cut, made no selection and ate whichever of the two substances came handy. However, those that started with wool nibbled a small quantity and having satisfied, so to say, the bare, immediate, and pressing need for water turned away and having discovered the green leaf, ate it to their fill. This occurred in all the four desiccators and showed very clearly that the real need for nourishment was met only by eating the leaf, while soaked wool was eaten just to appease an irresistible craving for water.

(iv) In another experiment the fliers were again kept in a dry atmosphere, as before. They were provided with some dry bran, dried fig leaves and dried cabbage leaves. After forty hours it was discovered that the dried fig leaf had been slightly nibbled, but the dried cabbage leaf and bran had remained untouched. At this stage some soaked wool was introduced into the desiccator. This was eaten very readily, although, as before, the quantity eaten was not much.

It is safe to conclude once again that it was not hunger but thirst that drew locusts to wet wool. Thus it is really the desire for water that makes the locust eat such a 'non-edible' substance as wool. This hypothesis receives further support from the following experiment:

(v) Locusts were kept at 33°C. and at about ninety-eight per cent relative humidity, for forty-eight, sixty, seventy-two, eighty-four and ninety-six hours. No green food was provided. They were supplied with a weighed quantity of wool soaked in water. In all cases the wool was not eaten. Some of the fliers that were kept for eighty-four to ninety-six hours died without touching the moist wool.

The reason may be that since the percentage of humidity in these experiments was high*, the loss of water from the bodies of locusts was low, and there was no desire for water. Therefore, in the absence of thirst, the locust refused to eat moist wool, though this was greedily eaten by the locusts kept in an atmosphere with low humidity.

Nikolsky [1925] has quoted instances of the Asiatic Locust marching on a line upon which linen was spread for drying. They ate up the wet ones and left untouched those that had dried. This again is a proof for the hypothesis put forth. The record already mentioned of locusts eating wool from living sheep also finds a satisfactory explanation on the basis of this hypothesis. Obviously, the wool on their body must have been moist.

In this connection another observation of Nikolsky is of interest. He observed a band of hoppers of the Asiatic Locust marching in a desert tract. Having met a pond of water they concentrated on it and it was clearly seen that they were drinking water. In this laboratory also, sometimes, during hot dry days, the Desert Locust has been seen drinking water sprinkled on the soil of the rearing cages.

IV. Discussion

A certain amount of water in the tissues of an organism is absolutely necessary for its life. For the maintenance of this minimum the beasts of the desert have to make special efforts. A number of animals in the dry regions of the earth are known periodically to march across long stretches of sandy areas in quest of watering places. Birds of the desert go regularly to their watering places and bring back water for their young ones. Perhaps the most interesting contrivance for this is that of the Sand-grouse [Buxton, 1923]. They drink while standing in water with their breasts submerged and return home with the plumage on their breasts soaked in water. The young ones pass the feathers through their bills and keep on changing until the supply becomes exhausted.

Normally locusts get their supply of water through their food. When this is scarce and the loss of moisture from the body, in a warm and dry atmosphere, is excessive, a stage is reached, when, in order to live, the locust must obtain water from some other source. It is precisely under these conditions that locusts eat practically anything which can provide water.

In the deserts, during periods of drought when vegetation is parched, the vegetable-eating insects meet their demands for water by eating pieces of dry plants. In sandy deserts, while the days are excessively hot and dry, the nights are cool and the humidity is then very high. The diurnal range of humidity is very wide. Buxton [1924] has observed that often when the humidity during the day is as low as twenty per cent, during the night the air is nearly or quite saturated. Desiccated fragments of certain plants situated in an atmosphere containing eighty per cent humidity or more, absorb an amount of moisture equal to fifty to sixty per cent. of their own dry weight (Buxton). It

* Saturation deficiency below 1 mm.

is evidently on account of this moisture that a variety of apparently dry articles are eaten by the locust. In the case of wool on the body of the sheep, in addition to the small amount of hygroscopic moisture, it is likely, that a fair amount of free moisture should also have been existent.

It may be mentioned here that in the case of swarming hoppers the problem of water economy is heightened by their colour. It is well known that black absorbs more radiant heat than any other colour. Thus under the same climatic conditions a swarming (black) hopper will absorb more heat than a solitary (green) one and will therefore require more water for evaporation in order to keep down its temperature at the optimum, than a solitary (green) hopper would require. [In most animals the temperature of the body is brought down by evaporation of water from the body surface. Among insects this is done by evaporation through respiration (Buxton)]. Therefore, the swarming hoppers stand in greater need of moisture.

It can thus be safely concluded that sometimes even non-edible substances are eaten by locust to make up the amount of water required for its existence. A study of the water requirements of locust under different conditions is intimately connected with the problem of locust control by means of poison baits. A dry bait is entirely useless and the failure of baiting in certain places may be attributed to this fact. Locusts need water, particularly in a dry atmosphere and this is exactly the atmosphere in which the bait would dry very quickly. Thus baiting cannot be employed by mere rule of thumb. One must study the environmental conditions before a proper control through this method can be obtained.

V. Summary

Non-edible substances, like wool, are eaten by the Desert Locust to obtain moisture, such substances are not eaten if they are dry.

Substances containing a large amount of hygroscopic moisture are also eaten.

The tendency is particularly marked if the atmosphere is dry.

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NITROGEN RECUPERATION IN THE SOILS OF THE BOMBAY PRESIDENCY, PART IV

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CONTENTS

	Pages
I. Introduction	268
II. Nitrogen fluctuations in non-irrigated soils	276
III. Nitrogen fluctuations in soils under irrigated conditions	281
IV. Nitrogen fluctuations in soils which are irrigated and are under crop all the year round	288
V. Nitrogen fluctuations in soils under heavy rainfall conditions	294
VI. Discussion of results	298
VII. Conclusions	311
References	311
Appendix	313

I. Introduction

Historical

The investigation of the nitrogen recuperation in the soils of the Bombay Presidency was started as long ago as 1923 and the first results were published as a memoir with the title " Nitrogen Recuperation in the Soils of the Bombay-Deccan,

Part I" [Sahasrabuddhe and Daji, 1925]. The experiments were conducted in the laboratory on samples of the medium black soil of the Deccan, taken at the end of the hot weather, kept dry and then exposed to varying conditions. The results showed that a good deal of nitrogen fixation takes place in the trap soils when they are moistened and kept at suitable temperatures. Further work on this subject was published in this *Journal* as " Nitrogen Recuperation in the Soils of the Bombay Presidency, Part II " [Sahasrabuddhe and Ghatikar, 1931]. For the investigation published in this paper, typical soils of the Bombay Presidency were obtained and the effects of the additions of lime, phosphatic substances, organic matter and alkali salts were studied. The results proved that at least under the controlled conditions of the laboratory, the addition of lime, phosphatic substances and organic matter improved the nitrogen recuperation of such soils as are not originally rich in these ingredients.

It remained, however, to be seen whether any recuperation takes place actually under field conditions. This investigation was undertaken soon after the publication of Part II and the results were published in this *Journal* [Sahasrabuddhe and Kanitkar, 1932]. Those results show that definite recuperation of nitrogen takes place in the soils under field conditions in the dry-farming tracts. Addition of organic matter increases the recuperative power of the soil. The quantities of nitrates also fluctuate between certain limits.

Since the publication of Part III, further work on the fluctuation of the nitrogen contents of the soil has been done by other investigators both in India and in other countries.

Prescott and Piper [1930] find that the activity of nitrification is greatest in surface layers which are cultivated, during the process of fallowing. There is evidence that the high nitrate accumulation is in late spring in spite of rapidly falling of moisture contents, because this is associated with the higher temperatures prevailing at these periods. Nitrate accumulation occurred rapidly with dry soil although more slowly than under the optimum condition (17 per cent moisture).

Penman and Rountree [1932] found that there was no appreciable interference with nitrate accumulation in cropped soils during the early life of the crop. At later stages comparatively very poor capacity for accumulating nitrate was observed in the cropped soils. This led to a disadvantage, in the case of cropped soil as compared with bare soil at harvest time.

The important results obtained by Bartholomew [1932] show that the accumulations of nitrates in the uncropped soils has an important practical significance. It shows very forcibly the necessity of keeping the ground covered with some kind of crop even though it is only weeds. The cover crops prevent loss of large quantities of available nitrogen and in addition add considerable active organic matter to the soil when ploughed in.

Nitrification processes take place at a minimum soil temperature of 40°F. if the soil conditions are favourable. Consequently, after crops have stopped growing, nitrification takes place and nitrates may accumulate in the soil before cold weather sets in.

Rheinwald [1933] in his paper on "Nitrate concentration of the soil solution and factors determining it," states that high temperature and moderate rainfall are important factors in the production of high nitrates in the soil solution. Heavy rains may seriously deplete the soil solution in the surface layer of nitrates and *vice versa*. Cropping tends to minimise variation in nitrates.

Desai [1933] in his experiments on "The influence of green manure and organic residues on nitrogen fixation in soils" found that fixation of nitrogen definitely takes place when green manure, straw or any other organic material is added to the soil under favourable conditions of temperature and moisture. Whether the crop is leguminous or non-leguminous the total nitrogen of the soil is brought up to the same level, because, although the leguminous plants are much richer in nitrogen at the time of addition to the soil, the subsequent nitrogen fixation in case of non-leguminous crop is much greater. The aeration of soil proved to be the main factor responsible for nitrogen fixation.

Garlach [1934] tried experiments on nitrogen fixation on artificially prepared soil by mixing sand, Kaolin, ground marble and the various salts with a small quantity of soil in suspension. From the results obtained by him it appears doubtful to him whether any free nitrogen fixation takes place in soils which are humus-free or which contain only small amount of organic matter. Such nitrogen fixation takes place in soils to which dextrose has been added as a source of energy for bacteria.

Vandecaveye and Villanueva [1934] found that "the quantities of nitrogen fixed during the course of the experiment varied with the organic matter content of the soils. Nitrogen fixation was greatly stimulated in three of the four soil samples which had received large amounts of carbonaceous organic residue."

Wilshaw [1934] observes that his experiments show that the total nitrogen content of the Malayan soil is subject to fluctuations which are too high to be accounted for by loss as soluble humate. He further says that one is forced to the conclusion that the enormous losses are mainly in the form of gaseous nitrogen. To counteract these losses, large recoveries are taking place producing the fluctuations observed. This can only mean that rapid fixation is occurring at certain periods.

Sreenivasan and Subrahmanyan [1934] state that it may be noted that there was considerable fluctuation in nitrogen similar to those recorded by some previous workers. On the other hand, the observations on the parallel plots show absolutely no relation to changes in season. The quantity of nitrates present at any time

shows no relation to either season or treatment. They further state that although it is desirable to study changes in the different soils under conditions actually obtaining in field practice, there are yet serious inherent defects due to soil heterogeneity, etc.

The plan of work

The experiments in connection with recuperation of nitrogen under field conditions published in Part III, were carried out on soils which were brought under cultivation only a few years before the experiments were started. They were virgin soils up to 1924, occupied by scrub jungle and *babul* shrubs. There *juar* was taken as a *rabi* crop.

The present investigation was started to find out what fluctuations in soil nitrogen take place under other conditions of climate and soil treatment, especially the fluctuations of nitrogen in soils under irrigation and under heavy rainfall.

The experiments were conducted at three places—(1) Poona, (2) Belgaum and (3) Karjat. Each place has its own peculiar climatic conditions and soil treatments.

In Poona there were two sets of experimental plots, *viz.* one set of plots for *kharif* (rainy season) *juar* and the other set for turmeric under irrigation.

For the *juar* crop a small area was divided into two adjacent plots measuring 55 ft. × 20 ft. each. In one of them the crop was sown and the other was left uncropped but underwent the same cultural operations as the cropped plot.

Another area was selected for the irrigated crop in order to study the nitrogen fluctuations under conditions of irrigation. This was divided into two equal adjacent plots measuring 110 ft. × 10 ft. each. Turmeric was sown on the cropped plot and the adjacent uncropped plot received the same manure, irrigation and other operations as the cropped plot.

The experiments at Belgaum were conducted on a soil the constituents of which are derived from both trap and laterite rocks. The soil differs much from that of Poona. The climatic conditions also differ. The peculiarity of the tract is that three crops are taken in one year. Here an area was divided into three adjacent plots measuring 33 ft. × 33 ft. each. The first one was cropped throughout, the second was uncropped throughout but received all cultural operations including manuring and irrigation, while the third was left uncropped and received all cultural operations including irrigation but did not receive any manure.

At Karjat the soil is derived from trap. The tract received heavy rainfall from June to October. Rice is the crop taken from June to the middle of November. A suitable piece of land was divided into two adjacent plots measuring 33 ft. × 33 ft. each of which one was cropped and the other uncropped.

It will be noticed that in each case it was a single-plot experiment. As it was not possible to do the experiment in replicated plots, the difference between the individual figures as distinct from the general trend may be taken with reserve.

Chemical and mechanical analyses

TABLE I

Chemical analysis

	Poona *juar* plot	Poona turmeric plot	Belgaum laterite plot	Karjat trap soil plot
	On fine air-dry matter			
	Per cent	Per cent	Per cent	Per cent
Moisture	7·53	6·54	3·18	5·37
Loss on ignition	7·45	8·00	11·02	7·17
Sand (acid-insoluble)	62·46	65·47	49·61	61·86
Iron (Fe_2O_3)	6·49	6·34	17·58	11·40
Alumina (Al_2O_3)	6·57	5·72	16·64	11·26
Lime (CaO)	6·17	4·50	1·05	0·92
Magnesia (MgO)	2·00	1·82	0·98	2·36
Potash (K_2O)	0·36	0·36	0·55	0·22
Phosphoric acid (P_2O_5)	0·054	0·06	0·36	0·11
Organic matter (direct determination)	1·53	2·16	4·44	1·81
Total water-soluble salts	0·044	0·13	0·14	0·035
	Grams			
The maximum water-holding capacity of soil showed that 100 grams of oven-dry soil could take up given grams of water	78·32	71·29	75·91	67·30

TABLE II

Mechanical analysis

(By International Soda Pipette method. The soil was passed through one mm. sieve)

	Poona *juar* plot soil	Poona turmeric plot soil	Belgaum laterite plot soil	Karjat trap soil
	On fine air-dry matter			
	Per cent	Per cent	Per cent	Per cent
Moisture	7·53	6·54	3·18	5·37
Coarse sand	12·07	11·25	11·22	4·53
Fine sand	27·88	30·65	14·32	24·99
Silt	25·05	20·30	18·60	30·42
Clay	24·99	25·17	47·24	27·77

pH values

pH values for all the soil samples were determined by a potentiometer with a quinhydrone electrode.

Method of taking samples

Samples were taken by randomisation. From each plot twelve samples of surface soil of six in. depth were taken and thoroughly mixed up and a composite sample was taken, dried at 60° C. to prevent changes and then brought to Poona. Soil sample for moisture was taken separately. The randomised spots from which the samples were taken were distributed fairly evenly over the whole plot as will be seen from the detailed description later on.

The sampling error

There is a likelihood of error in collecting samples even though these are taken by randomisation. In order to find out the limits of this error, soil samples were collected from uncropped *juar* plot. Twelve samples were taken each time by randomisation and this was repeated six times on one and the same day. This

gave six composite samples, each containing twelve samples as usual. The figures for total nitrogen were as follows :—

TABLE III

Total nitrogen in mgs. per 100 grms. of oven-dry fine soil

No. of composite sample of soil	Total nitrogen
	(Mgs.)
1	44·52
2	44·05
3	44·09
4	43·93
5	44·36
6	44·02

The standard deviation of these comes to 0·228. This is very small indicating that the method of collecting samples is satisfactory and the differences in the nitrogen contents of the samples need not be put down as due to error in sampling.

Soil temperatures were taken at 5 P. M. on the days on which samples were taken.

Methods of analysis

Total nitrogen.—Twenty grams of soil passed through one-mm. sieve, was taken in a Kjeldhal flask. The soil was moistened with distilled water and 50 c. c. of salicylic-sulphuric acid (one grm. in 30 c. c. of sulphuric acid) was added to it and kept overnight. Next day five grams of sodium thiosulphate and two to four grams of potassium sulphate were added. To this mixture 20 c.c. of salicylic-sulphuric acid were added again. It was digested as usual and distilled. The distillate was collected in 0·05 *N* sulphuric acid and then titrated against 0·05 *N* caustic potash. Indicator used was alizarine sodium sulphate, which gives a sharp change.

Ammoniacal and nitrate nitrogen

One hundred grams of soil, passed through one-mm. sieve was shaken for half an hour with 150 c.c. of normal sodium chloride, filtered through Buchner funnel and then leached with 350 c. c. of normal sodium chloride solution. The whole filtrate was transferred to a distilling flask. Three grams of magnesium oxide was added and distilled in 0·02 *N* sulphuric acid. The distillation continued till the distillate collected was 175 c. c.

To this flask three grams of magnesium oxide were again added with three grams of powdered Devarda's alloy and distilled in 0·02 *N* sulphuric acid till the distillate collected was 175 c.c. Both these distillates were titrated against 0·02 *N* caustic potash separately with alizarine sodium sulphate as an indicator.

Special precautions taken in analysing the samples

(*a*) Investigators often take five to ten grams of soil sample for nitrogen determination. Twenty grams of soil were taken in this case to minimise errors in the final figures which increase by multiplication.

(*b*) 0·05 *N* sulphuric acid was used for total nitrogen.

(*c*) 0·02 *N* sulphuric acid was used for nitrate nitrogen estimation [Crowther and Mirachandani, 1931] to make titration as accurate as possible.

(*d*) Blank determinations were done and corrections made accordingly.

(*e*) Glass-distilled water was used all through.

All these precautions were taken to minimise the working error as much as possible.

Working error

Whatever the precautions one may take, there is sure to be some working error in the case of soil nitrogen which is present in small quantities. It is essential that working error is determined to get an idea of the extent of real fluctuation after making necessary allowance for all sorts of errors. Soon after the work was started, a soil sample was collected by randomisation of the spots, etc., on a plot at Karjat. This composite sample was divided into eight parts and soil from each of these was analysed for total nitrogen, ammoniacal nitrogen and nitric nitrogen. The figures obtained were as follows :—

TABLE IV

Milligrams of nitrogen in 100 grms. of oven-dry soil

Serial No.	Total nitrogen	Ammoniacal nitrogen	Nitric nitrogen
	(Mgs.)	(Mgs.)	(Mgs.)
1	77·52	0·64	0·14
2	77·52	0·66	0·14
3	78·38	0·64	0·14
4	77·98	0·68	0·16
5	77·75	0·64	0·16
6	77·75	0·68	0·14
7	78·38	0·68	0·12
8	78·18	0·64	0·12

The standard deviation for total nitrogen comes to 0·3527. Any difference which is more than three times the standard deviation, *i.e.* 0·3527 × 3 = 1·0581, is significant. Similarly for ammoniacal nitrogen any difference beyond 0·054 and for nitric nitrogen any difference beyond 0·045 will be significant.

Climatic data

Records of climatic data for all the stations at which experiments were carried on are given in the Appendix.

II. Nitrogen fluctuations in non-irrigated soils

The field experiments on nitrogen recuperation given in Part III (1932) were laid out at Manjri on a soil which was newly brought under cultivation, which was rich in nitrogen (0·073 per cent) and which received agricultural operations for *rabi juar*. For the purposes of this investigation another set of conditions was taken. The land selected was under cultivation for years, and contained comparatively a small proportion of nitrogen (0·041 per cent). It was without manure for years and was treated for a *kharif* crop of *juar*. The area was taken on the Poona Agricultural College Farm which was divided into two adjacent plots measuring 55 ft. × 20 ft. each. Both of these received the same operations from beginning to end, with the difference that one of them was cropped and other was not cropped.

Table V

Operations	Date
Ploughing by C. T2 plough 7 in. to 8 in. deep	18th March 1933
Discing by a disc harrow	22nd March 1933
Discing by a disc harrow	2nd May 1933
Harrowing by a blade harrow	3rd May 1933
Harrowing by a blade harrow	20th June 1933
Sowing by a country drill 12 in. apart	30th June 1933
Harvesting	6th December 1933

The previous crops on these plots were maize (*kharif*) and gram (*rabi*) in 1931-32 and *nilwa* (*kharif*) in 1932-33.

The depth of the soil was about two feet. The first samples of soils in connection with determination of nitrogen fluctuations in these plots were taken on the 30th of June and the subsequent samples were taken at the end of every month.

All the analytical figures are expressed on fine oven-dry soil. Every month twelve samples from each plot were taken by randomisation of spots and a composite sample of these was dried at 60° C. and taken to the laboratory. The accompanying chart (Fig. 1) will give an idea as to how the twelve spots for soil samples were distributed. Spots are shown for the month of December 1933 only as these are enough to show the distribution without causing confusion.

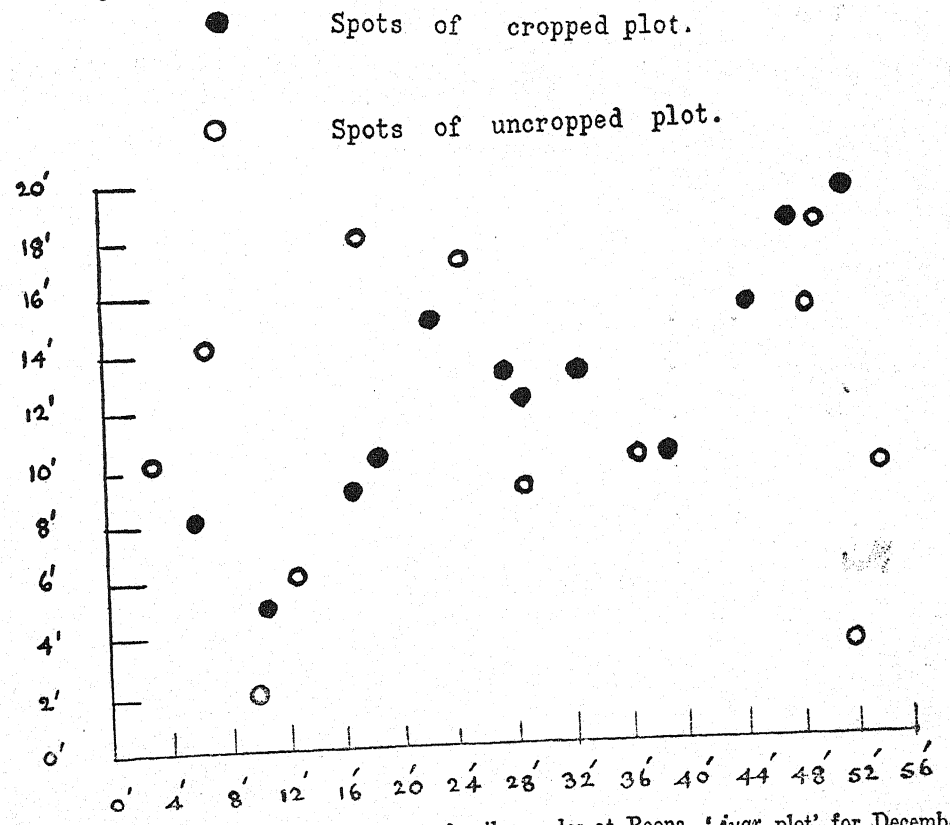

Fig. 1. Distribution of the twelve spots of soil samples at Poona '*juar* plot' for December 1933.

(*1*) *Nitrogen fluctuations on a plot on which* kharif juar *was grown.*

Total nitrogen and nitric nitrogen were determined from June 1933 to July 1934 to get one overlapping month. The following table gives total and nitric nitrogen along with the moisture contents of the soil. Soil temperatures were always taken at 5 P. M. on the days on which samples were collected. These temperatures may be useful as they are of the soil itself. It is quite possible that the nitrogen fluctuation may be affected by the minimum and the maximum temperatures of the whol

TABLE VI

Soil temperature, moisture, pH values, total and nitrric nitrogen in mgs. per 100 grms. of oven-dry fine soil

Time	Soil temperature	Soil moisture	pH value	Total nitrogen	Nitric nitrogen
1933					
Last week of—	°C.	Per cent		Mgs.	
June	..	33·83	8·30	51·07	0·21
July	..	41·05	8·39	43·38	0·10
August	..	31·01	8·33	48·52	0·11
September	33·6	31·86	8·23	56·67	0·20
October	28·1	26·54	8·20	49·22	0·58
November	29·17	29·63	8·37	47·82	0·45
December	29·6	26·86	8·19	54·04	0·64
1934					
January	31·8	21·52	8·32	47·15	1·49
February	32·0	17·80	8·29	53·81	0·96
March	36·0	12·02	8·27	54·44	0·31
April	37·9	8·86	8·34	52·91	0·15
May	39·3	8·71	8·14	52·37	0·29
June	26·0	29·45	8·38	50·92	0·36

It has already been shown that any difference which is more than 1·057 milligrams is significant. There is therefore no significant difference between the total nitrogen of February and March and also between that of April and May. But the fluctuations in total nitrogen for other months are significant. There is a sudden fall from 51·07 to 43·38 milligrams per 100 grams of oven-dry fine soil between June and July samples. The July sample was collected on the 28th of

July. Just previous to that date there was a rainfall of 4·62 inches (Appendix A). This must have washed away nitrogen to a certain extent and hence the lower figures for July. High figures for nitrogen are obtained in September, December and March. In September, although the crop is growing, there is a high proportion of water (not too much as in July) in the soil and the temprature also is high. This explains why the nitrogen contents are high in September. From November onwards the nitrogen contents rise although the water-contents and the temperatures slowly go down. This is because the crop which feeds on the nitrogen is removed. In January the nitrogen figure is low because the minimum temperature of that month is as low as 8 to 10°C. From February onwards, although the temperature rises, the moisture goes down and hence the two factors working against each other minimise fluctuations. The nitrogen figures for June 1933 and June 1934 do not differ from each other significantly. This clearly proves once again as shown by Sahasrabuddhe and Kanitkar [1932] that the total nitrogen of a soil is not constant but goes on increasing and decreasing forming a sort of an annual cycle. When a crop like *juar* is grown without manure but with all the necessary cultivation, the soil is able to recuperate all the nitrogen and this cycle may go on year after year under the climatic conditions of the Bombay-Deccan. The maximum fluctuation is from 43·38 to 56·67 milligrams per 100 grms. of oven-dry fine soil, *i.e.* a maximum fluctuation of 30·6 per cent. This does not mean that the soil becomes richer in nitrogen by 30·6 per cent at the end of the year but it shows the range of rise and fall and how the nitrogen contents of the soil are kept up from year to year.

The pH values of the cropped plot vary only but slightly, ranging from 8·16 to 8·39. The values are slightly lower in the months of September, October, December and May than in other months.

The pH values in the uncropped plot range from 8·16 to 8·53, showing a little greater range than in the cropped plot. The pH values for July, September, October, January and February are somewhat lower than for other months.

Nitric nitrogen as given in Table VI above shows a great fall from June to July on account of the heavy rainfall just before July sample was taken. After that there is a steady rise till January. In December, January and February the nitric nitrogen is high and then goes down. It would have fallen in June 1934 if the rains at that time had been as heavy as in June 1933.

(2) *Nitrogen fluctuations on an uncropped plot adjacent to the* juar *plot.*

As already stated, the uncropped plot received all the cultivation treatments as the plot under *juar* and any differences that may be observed are due to the absence of the crop. Table VII gives all the important figures.

TABLE VII

Soil temperature, moisture, pH values, total and nitric nitrogen in mgs. per 100 grms. of oven-dry fine soil

Time	Soil temperature	Soil moisture	pH value	Total nitrogen	Nitric nitrogen
1933					
Last week of—	°C.	Per cent.		Mgs.	
June	..	33·97	8·30	51·07	0·21
July	..	43·42	8·18	46·38	0·31
August	..	33·12	8·37	44·01	0·34
September	33·5	32·59	8·31	43·84	0·28
October	31·8	24·54	8·16	44·58	0·46
November	30·17	29·75	8·18	46·20	0·43
December	28·80	27·10	8·25	49·13	0·69
1934					
January	32·40	20·68	8·22	46·54	0·78
February	32·25	15·90	8·23	44·39	1·00
March	36·20	12·30	8·49	41·27	0·51
April	38·20	8·90	8·48	42·59	0·34
May	38·70	9·20	8·53	41·22	0·35
June	26·00	33·34	8·53	42·82	0·41

The total nitrogen contents for August, September and October are not significantly different. All the other figures show significant fluctuations. There is a fall from June to September and a rise from September to December. Then again there is a fall with a small fluctuation till May, and then a small rise. The highest figure is 51·07 mgs. in June and the lowest in May 41·22 mgs. Therefore the maximum fluctuation is 23·1 per cent.

The nitric nitrogen is high in December, January and and February as on the cropped plot. Except in the months of October, November and January, the nitric nitrogen is higher in the uncropped than in the cropped plot.

The nitrogen in the cropped and the uncropped plot was the same to start with, as the previous treatment of the plot was exactly the same. Later on the total nitrogen is lower in the uncropped plot than in the cropped plot throughout the period of the experiment. At the end of the year the uncropped plot is below the cropped plot in nitrogen. When the crop is removed the roots and fallen leaves get incorporated into the soil and thus add to the organic matter, but on the uncropped cultivated land nothing of that sort happens and hence the lowering of nitrogen in the uncropped plot. This confirms the results obtained by Sahasrabuddhe and Ghatikar [1931], Sahasrabuddhe and Kanitkar [1932], and also proved by Desai [1933], Penman and Rountree [1932], Vandecaveye and Villanueva [1934] and others that organic matter helps fixation of nitrogen. This means that if a land is cultivated it should be cropped. If it is left uncropped its nitrogen content is lowered.

III. Nitrogen fluctuations in soils under irrigated conditions

In Part III [Sahasrabuddhe and Kanitkar, 1932] and in section II of this paper experimental results are discussed for nitrogen fluctuations in non-irrigated soils. But there are lands on which irrigated crops are grown and it is therefore important to ascertain what sort of nitrogen fluctuations take place under irri ation conditions. A suitable plot was taken on the Poona Agricultural College Farm and divided into two plots each measuring 110 ft. × 10 ft. side by side with each other. A crop of turmeric was put on, one with the usual cultivation operations, manuring and irrigation, and the other plot was without a crop but received cultivation, manuring and irrigation as the first one. The weeds that came up were removed along with the weeding of the cropped plot. Nitrogen determinations were done from month to month by obtaining a composite sample from the twelve spots taken by randomisation. The distribution of the spots is shown in the accompanying chart (Fig. 2) for the month of April 1934 only. Beside these monthly samples taken for thirteen months other samples were taken from both, the cropped and the uncropped area from two depths 0 in. to 6 in. and 6 in. to 9 in. to see the difference in fluctuations of nitrogen in the two layers. All these determinations were carried out with the idea of getting the exact effect of irrigation.

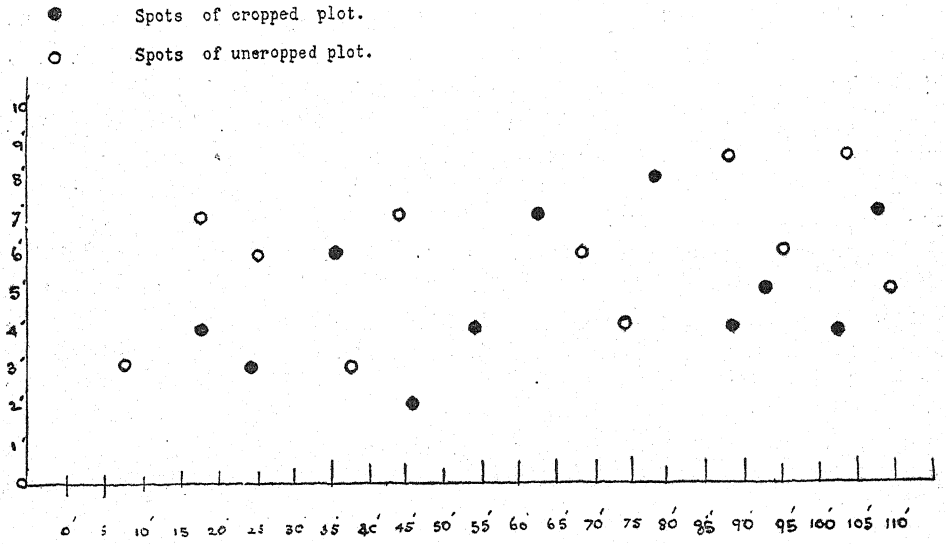

Fig. 2. Showing the distribution of the twelve spots of soil samples at Poona 'turmeric plot' for April 1934.

The soil of these plots is about a foot to one and a half foot deep. It is richer in organic matter and nitrogen than the soil for *juar* plots dealt with in the previous section.

As the irrigated plots were on the College Farm, the rainfall, temperature and humidity tables for these plots are the same as those for *juar* plots given in Appendix A. The cultivation and other operations including irrigation are given along with their dates in Table VIII.

TABLE VIII

Operations	Date
Ploughing by C. T. plough 7 in. to 8 in. deep	23rd March 1933
Manuring with farmyard manure at 20 cart-loads per acre. Discing by a harrow	24th May 1933
Preparing broad ridges 5 ft. apart by Victory plough.	25th May 1933
Planting sets at 12 in. × 12 in. apart	26th May 1933
Watering	27th May 1933
Weeding	21st June 1933
Watering	11th July 1933
Weeding by hand	18th August 1933
Top dressing at the rate of 62 lbs. nitrogen per acre, half in the form of sulphate of ammonia and half in the form of groundnut cake powder	7th October 1933
Watering approximately at intervals of ten days from	28th October 1933 to 20th February 1934
Weeding by hand	18th November 1933
Weeding by hand	1st December 1933
Harvesting	12th March 1934

Operations during 1934

Ploughing with C. T. 2 plough . . . . . 23rd April 1934
Clod crushing with Norwaygian harrow . . . 24th May 1934

On the turmeric plots the previous crops were maize (*kharif*) and gram (*rabi*) in 1931-32 and *shalu juar* (*rabi*) in 1932-33.

Table IX gives the pH values, the moisture-contents and total nitrogens of the cropped and uncropped irrigated plots; the temperatures of the soils of the two plots at the time of taking samples did not vary beyond 1°C. and hence the temperatures of the cropped plot only are given.

TABLE IX

Soil temperature, moisture, pH values, total nitrogen in mgs. per 100 grams of oven-dry fine soil

Time	Soil temperature	Cropped			Uncropped		
		Soil Moisture	pH value	Total nitrogen	Soil moisture	pH value	Total nitrogen
1933 Last week of—	°C.	Per cent		(Mgs.)	Per cent		(Mgs.)
June	..	26·00	8·19	87·25	27·11	8·19	87·25
July	..	25·92	8·48	75·11	27·67	8·28	82·65
August	..	22·11	8·30	70·59	20·87	8·42	80·63
September	..	21·79	8·35	72·07	21·63	8·32	76·34
October	..	16·33	8·30	77·84	16·67	8·32	76·91
November	27·0	21·84	8·27	76·33	24·98	8·17	77·15
December	28·5	21·27	8·30	84·03	22·03	8·42	83·02
1934 January	28·1	19·40	8·43	77·79	17·85	8·42	83·66
February	30·7	19·89	8·44	73·73	20·15	8·36	93·51
March	37·4	5·82	8·42	71·72	6·43	8·34	90·15
April	38·0	5·53	8·28	82·20	6·47	8·49	78·96
May	39·8	5·20	8·37	75·27	6·37	8·40	69·11
June	28·6	25·72	8·45	75·69	24·18	8·45	73·24

The moisture in the uncropped plot in most cases is higher than in the cropped plot both during the rains and after.

In the cropped plot the fluctuation from May 1934 to June 1934 is not significant but all other fluctuations are significant. In May 1933 the plots received organic matter in the form of farmyard manure and there was rain in May and June. The soil sample for June 1933 therefore shows a high nitrogen figure. It goes down rather rapidly in July on account of the heavy rainfall (4·66 inches) just before the soil sample was taken. There is a further fall in August, a steady rise till December and a fall till March. When the crop is harvested in March, there is a rise in nitrogen contents in April on account of the removal of the crop. A fall soon starts as the soil contains but very little moisture. The sample of June 1934 was expected to come up to the level of the sample of June 1933 but the conditions were unfavourable.

In 1933, after the addition of the farmyard manure in May, there was good rainfall both in May and June. In 1934, however, there was no rain in May and farmyard manure was added as late as 15th June.

In the uncropped plot there is no significant difference between the total nitrogen figures for September and October and between the figures for October and November. The same is the case between the figures for December and January. All other fluctuations are significant. The nitrogen decreases from June to September. It remains at a low level during September, October and November and goes on increasing till February. From March onwards the irrigation is stopped, the soil moisture goes down very much and the nitrogen naturally decreases, falling to its minimum in May.

The difference between the irrigated cropped and irrigated uncropped plots is that the nitrogen is low in the cropped plot during the period the crop is growing, but when the crop is harvested the cropped plot shows more nitrogen than the uncropped one. After the crop is removed the organic matter in the form of roots, etc., which is left behind in the cropped plot, perhaps helps nitrogen fixation.

The maximum fluctuation of total nitrogen in the cropped plot is from 70·50 to 87·25 mgs. *i.e.* 23·6 per cent. In the uncropped plot it is from 69·11 to 93·51 mgs. *i.e.* 35·3 per cent.

The addition of 62 lbs. of nitrogen per acre as manure on the 7th of October makes but little difference, as when the quantity added is distributed over six inch acre, it comes to about 3 mgs. per 100 grms. of soil for October sample. But the variations from month to month are more than 3 mgs. before October and even after October.

There are slight of fluctuations in the pH values but there is no definite change in any direction in the cropped or in the uncropped plot.

The nitric nitrogen of the two plots under irrigation is given below :—

TABLE X

Nitric nitrogen in mgs. per 100 grams of oven-dry soil

Time	Cropped	Uncropped
1933		
Last week of—	(Mgs.)	(Mgs.)
June	0·43	0·43
July	0·49	0·33
August	0·21	0·27
September	1·10	0·81
October	2·23	1·13
November	1·34	3·51
December	2·63	3·91
1934		
January	2·29	3·62
February	2·17	2·83
March	1·72	4·37
April	1·86	2·29
May	1·40	1·58
June	2·32	3·46

Nitric nitrogen goes down till August. It then rises giving the highest figure in December for the cropped and the uncropped although there is another higher figure in the uncropped in March. The nitric nitrogen is less in the cropped than in the uncropped plot in most of the months.

The nitric nitrogen in June 1934 is much higher than that in June 1933 in both the cropped and the uncropped plots. This is perhaps because in the year 1933 there was a rainfall of 11·41 inches in May and June before the sample was taken and hence the nitrates were washed away. In May and June 1934, however, the rainfall before the soil sample was taken amounted only to 4·18 inches distributed all over the month and the nitrates were not washed away as in June 1933. When the July rains came, the total rainfall for May, June and July of 1934 went

up to 8·31 inches. Nitrates got washed and the July figures for the nitrates in the soil came down as shown below :—

TABLE XI.

Nitric nitrogen in mgs. per 100 grms. of oven-dry fine soil

Month	Rainfall before the sample	Cropped plot	Uncropped plot
	Inches	(Mgs.)	(Mgs.)
May and June 1933	11·41	0·43	0·43
May and June 1934	4·18	2·32	3·46
May, June and July 1934 . . .	8·31	0·66	0·56

(2) *Difference in the nitrogen fluctuations of the upper six inches and the lower three inches of soil*

The irrigation water was analysed from time to time but it did not give any nitrogen, and so the figures for nitrogen were not in any way affected by irrigation waters. In determining fluctuations in nitrogen contents only the upper six inches of soil were used. It was, however, considered necessary to ascertain whether the nitrogen fluctuations in the lower three inches have the same trend as the upper six inches and whether the lower three inches contain more total nitrogen than the upper six inches. This is very important because if the tendency is to concentrate nitrogen in the lower layer, to study the fluctuations merely in the upper six inches may not give a complete idea of the variation taking place, especially on irrigated land where the tendency to wash down constituents is likely to be more than on unirrigated land.

In order to ascertain this, on certain occasions, samples of soils from the upper six inches and also those of 6-in. to 9-in. just below the upper samples, were taken by the usual method of randomisationa and preparing composite samples. This set was done quite independently of the set of samples analysed from month to month.

These samples were collected on different dates between the 16th November 1933 and the 24th of February 1934 from the cropped and the uncropped irrigated plots for turmeric on the Poona Agricultural College Farm. Total nitrogen and nitric nitrogen were determined as usua, along with the moisture in the soil. The moisture varied from 19 to 22 per cent.

The following table gives total nitrogen in both the cropped and the uncropped irrigated plots.

TABLE XII

Total nitrogen in mgs. per 100 grms. of oven-dry soil

Serial No.	Dates of samples	Cropped plot		Uncropped plot	
		0-in. to 6-in.	6-in. to 9-in.	0-in. to 6-in.	6-in. to 9-in.
		(Mgs.)	(Mgs.)	(Mgs.)	(Mgs.)
1	16th November 1933 . .	70·85	65·96	76·05	67·38
2	24th November 1933 . .	67·03	60·01	90·63	85·70
3	1st December 1933 . . .	76·33	69·39	77·14	73·13
4	18th December 1933 . .	74·95	67·43	75·60	74·54
5	23rd December 1933 . .	79·85	70·67	88·59	72·85
6	27th December 1933 . .	84·04	63·15	83·03	70·23
7	9th January 1934 . . .	68·07	62·54	74·89	70·06
8	15th January 1934 . . .	77·03	68·08	75·28	64·76
9	24th January 1934 . . .	77·79	67·65	83·66	81·58
10	30th January 1934 . . .	69·74	71·15	94·21	82·58
11	5th February 1934 . . .	69·58	71·10	82·43	81·01
12	19th February 1934 . .	72·72	69·05	77·29	72·79
13	24th February 1934 . .	73·73	69·74	93·48	75·27

Table XII shows that the lower three inches generally contain less total nitrogen than the upper six inches and further that the trend of fluctuations is the same in the upper and lower layers. This shows that the inferences drawn by determining nitrogen fluctuations from the top six-inch layers are correct as far as the trend of total nitrogen fluctuations is concerned and also with regard to the recuperation of nitrogen by the soils.

Figures for nitric nitrogen from the irrigated cropped and uncropped plots are interesting. They are given in Table XIII.

TABLE XIII

Nitric nitrogen in mgs. per 100 grms. of oven-dry soil

Serial No.	Dates of samples	Cropped plot		Uncropped plot	
		0 in. to 6 in.	6 in. to 9 in.	0 in. to 6 in.	6 in. to 9 in.
		(Mgs.)	(Mgs.)	(Mgs.)	(Mgs.)
1	16th November 1933 . .	1·20	1·48	1·68	1·62
2	24th November 1933 . .	1·20	1·04	2·87	1·71
3	1st December 1933 . . .	1·34	1·40	3·51	2·28
4	18th December 1933 . .	1·67	1·44	2·13	1·50
5	23rd December 1933 . .	1·65	1·68	4·15	1·63
6	27th December 1933 . .	2·63	1·21	3·91	2·30
7	9th January 1934 . . .	1·74	1·14	3·25	2·07
8	15th January 1934 . . .	1·39	1·58	2·14	1·29
9	24th January 1934 . . .	2·29	1·80	3·62	2·54
10	30th January 1934 . . .	1·66	1·58	2·34	2·63
11	5th February 1934 . . .	0·92	1·42	2·50	3·60
12	19th February 1934 . .	2·11	2·85	2·54	2·43
13	24th February 1934 . .	2·17	1·48	2·83	2·21

These figures show that the nitric nitrogen in the cropped plot both for the upper six inches and the lower three inches of soil is invariably less than that in the uncropped plot. In the uncropped plot the nitric nitrogen in the upper six inches is generally higher than that in the lower three inches of soil. No such relation is seen in the nitric nitrogen of the cropped plot. The figures for the two layers do not differ much from each other.

IV. NITROGEN FLUCTUATIONS IN SOILS WHICH ARE IRRIGATED AND ARE UNDER CROP ALL THE YEAR ROUND

At Belgaum a rainfall crop of rice is followed by irrigated crops of vegetables. There is a system of taking three crops in a year and the land is cropped in the same manner year after year. There is the monsoon crop of rice from July to October. When the rice crop is removed the land is ploughed and ordinarily manured with farmyard manure at the rate of 7·5 tons per acre. Suitable beds are made for irrigation and a mixed crop of cabbage, knolkhol and potatoes is grown. This crop is on the land till the middle of April. A top dressing of ammonium sulphate (160 lbs. per acre), super-phosphate (112 lbs. per acre) and potassium sulphate (112 lbs. per acre) is given in the months of January and December. In the second half of April the land is harrowed and sweet potato cuttings are planted. This crop is removed in the form of cuttings in the month of June. The remaining portion is uprooted, the land is harrowed three times and prepared for drilling rice in July. The actual operations done on the plots under experiments are given in Table XIV.

TABLE XIV

Agricultural operations on the plots under experiment at Belgaum during 1933-34

Operations	Dates
Three harrowings.	In May 1933
Drilling of rice seed (90 lbs. per acre)	22nd June 1933
Light interculturing and *Pesati* operation	5th July 1933
Interculturing and *Pesati* operation	15th July 1933
Twice interculturing	1st August 1933 and 10th August 1933
Hand weeding	8th September 1933
Harvesting of rice	17th November 1933
Ploughing	18th November 1933
Manuring with farmyard manure (2½ tons per acre) and groundnut shell manure (7½ tons per acre). Harrowing and preparing beds for planting	12th December 1933
Planting of cabbage, etc., manuring with super-phosphate (112 lbs. per acre) and potassium sulphate (112 lbs. per acre)	15th December 1933
Well irrigation from 18th December to 9th June 1934 on every fourth day approximately	In all there were 29 irrigations given to the plots.
Weeding with *Fawadu* and manuring with sulphate of ammonia (160 lbs. per acre)	19th January 1934
Weeding and pruning of leaves	5th March 1934
Removal of knolkhol	7th April 1934
Weeding and planting of sweet-potato creepers	20th April 1934
Removal of cabbages	28th April 1934
Removal of cabbages	7th May 1934
Weeding by hand	13th May 1934
Removal of cabbages	29th May 1934
Removing of sweet-potato creepers	25th June 1934
Removing of sweet potato creepers. Removing of weeds, uprooting of sweet-potato creepers, harrowing crosswise and sowing of paddy	16th July 1934
Hand weeding	7th August 1934

The climatic data of Belgaum are given in Appendix B.

The soil at Belgaum on which the experiments were carried out is a mixture of material derived chiefly from laterite and partially from the Deccan trap. The soil is three feet deep and overlies laterite rock. It is well drained and is under cultivation for many years past.

A suitable piece of land was divided into three adjacent plots each measuring 33 ft. × 33 ft. Plot A was irrigated, manured and cropped ; plot B was manured and irrigated but had no crop, while plot C was only irrigated but had neither manure nor any crop. The samples of soils were collected from July 1933 to July

1934, at the beginning of every month. This cycle gives one overlapping sample for July. The collection of samples was done as already stated by randomising twelve spots for each plot, and taking composite sample by mixing twelve samples. Fig. 3 will give an idea as to how the twelve spots for soil samples were distributed for the month of June 1934 only. As all the three plots were under heavy rains and were irrigated every fourth day from the end of the rainy season of 1933 to the beginning of rains in 1934, the soil temperatures of the three plots taken on the same day did not vary beyond one °C. and the moistures not beyond one per cent. and hence in Table XV below, temperature and moisture of soil in plot A only are given to prevent unnecessary repetition. Only total nitrogen contents are taken in this table for three plots while nitric nitrogens are considered further on.

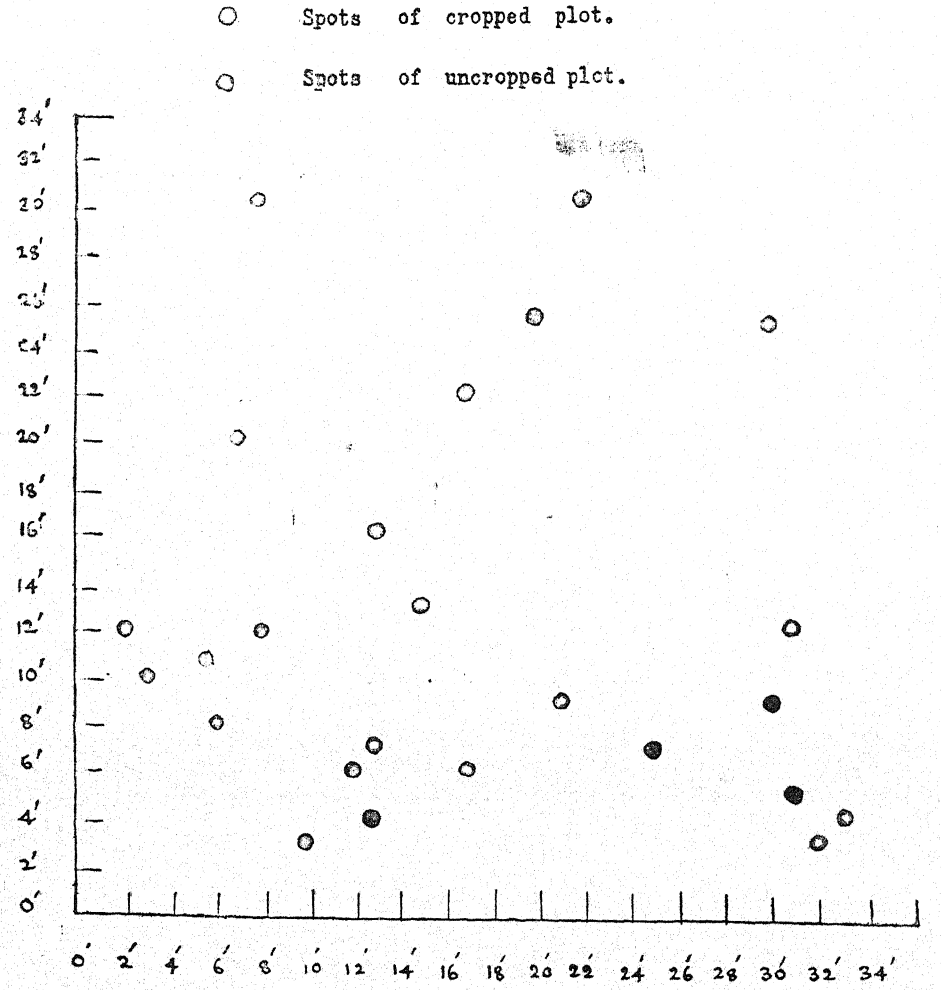

Fig. 3. Showing the distribution of the twelve spots of soil samples at Belgaum for June 1934.

TABLE XV

Soil temperature, moisture, pH values, total nitrogen in mgs. per 100 grams of oven-dry fine soil

Time	Soil temperature	Soil moisture	A		B		C	
			pH value	Cropped and manured	pH value	Uncropped and manured	pH value	Uncropped and unmanured
1933 First week of—	°C.	Per cent		(Mgs.)		(Mgs.)		(Mgs.)
July	..	28·65	7·99	205·09	7·99	205·09	7·99	205·09
August	..	43·16	8·01	213·57	8·12	225·19	8·08	200·46
September	..	43·16	7·83	242·89	7·76	221·74	7·89	212·93
October	26·0	34·28	7·32	240·19	7·46	225·00	7·78	236·47
November	26·5	34·21	7·85	256·94	8·04	211·84	7·94	216·15
December	23·7	34·34	7·94	216·18	7·93	233·32	7·89	204·07
1934 January	23·1	30·78	7·97	203·51	8·06	222·10	8·01	210·26
February	28·3	27·20	7·91	222·12	7·96	203·63	7·97	208·52
March	28·2	27·03	8·09	220·91	8·01	216·60	8·04	199·48
April	28·7	25·89	8·08	211·83	8·03	221·96	8·05	197·69
May	33·3	23·49	7·95	216·37	8·15	206·39	8·15	207·00
June	26·0	23·24	8·12	221·06	8·08	234·42	8·09	209·44
July	..	43·16	7·92	228·76	8·16	218·89	7·93	198·70

In considering the figures in Table XV three periods may be taken. The first from July to November when the rice crop is growing, no manure is added to any plot and there is no irrigation, but all the plots get rain water. The second period is from November to April. In this period all the three plots get well-irrigation. Plots A and B get organic manure in the beginning of December and plot A has vegetables like cabbage, etc., growing. The third period is from April to July when no manure is added, all the plots are irrigated and plot A has sweet-potato creepers growing.

During the first period the total nitrogen in all the plots increases. When the crop is growing usually the nitrogen in the cropped plot should be less than that of the uncropped plot as in the case of turmeric, but contrary to this the results of the plots at Belgaum show that when the rice crop is growing the nitrogen in the cropped plot is much higher than that in the uncropped plots.

The highest rise in plot A is in November. In plot B the rise occurs in December while in plot C it occurs in October. Soon after the rice crop is removed the total nitrogen in plot A falls and the total nitrogen figures of plot A are nearly the same as or even less than those of plot B. This may be due to the presence of vegetable crop on plot A as is in the case of turmeric plot at Poona. The unmanured plot C contains less of total nitrogen than the manured plot B practically all along. One striking fact comes out of the figures of total nitrogen given for plots A, B and C. The plot A with the rice crop growing on it shows more nitrogen than the uncropped plot while the same plot with vegetables growing on it shows less nitrogen than the plot without a crop. Plot C, which was neither cropped nor manured, shows less nitrogen in July 1934 than in July 1933. This is because it got no organic matter during 1934. Plot B, which was uncropped but got the same manure as plot A, shows more nitrogen in July 1934 than in July 1933. This is a distinct effect of the addition of organic matter in December 1933. The July 1934 figure for plot A, which was both manured and cropped, is higher than that for plot B. Plot B shows the advantage of manure over no manure and plot A shows the advantage of the cropped over uncropped.

The total quantity of manure added contains one milligram of nitrogen in 100 grams of soil in six inch acre. This is a very small quantity compared to the fluctuation in total nitrogen from month to month, before or after the manure was given.

The maximum fluctuation in plot A is from 203·51 milligrams per 100 grams of oven-dry, fine soil in January to 256·94 milligrams in November, which is 26·25 per cent.

The maximum fluctuation in plot B is from 203·63 milligrams per 100 grams of oven-dry, fine soil in February to 233·32 milligrams in December which is 14·58 per cent.

The maximum fluctuation in plot C is from 197·69 milligrams per 100 grams of oven-dry, fine soil in April to 236·47 milligrams in October which is 19·61 per cent.

The pH values of the cropped plot A vary from 7·92 to 8·18. They show a slight fall during the months of September, October and November and practically remain the same for the other months. The other plots behave very much in the same way. The range of the uncropped, manured plot B is from 7·46 to 8·16 while for the uncropped, unmanured plot C it is 7·78 to 8·15.

The quantities of nitric nitrogens of the same plots are given in Table XVI.

TABLE XVI

Nitric nitrogen in mgs. per 100 grams of oven-dry soil

Time	Soil temperature	Soil moisture	Cropped and manured	Uncropped and manured	Uncropped and unmanured
1933					
First week of	°C.	Per cent	(Mgs.)	(Mgs.)	(Mgs.)
July	..	28·65	0·51	0·51	0·51
August	..	43·16	0·33	0·42	0·48
September . . .	..	43·16	0·33	0·05	0·10
October . . .	26·0	34·28	0·39	0·51	0·57
November . . .	26·5	34·21	0·49	0·37	0·96
December . . .	23·7	34·34	0·34	0·35	0·64
1934					
January . . .	23·13	30·78	0·73	2·25	0·70
February . . .	28·25	27·20	1·45	0·99	1·09
March	28·16	27·03	0·95	1·37	1·31
April	28·70	25·89	0·93	4·10	2·82
May	33·30	23·49	0·75	2·69	2·74
June	26·0	23·24	3·82	4·88	6·01
July	..	43·16	0·35	0·55	0·52

One fact is clear from the above figures that after the rice crop is harvested the uncropped plots contain more of nitric nitrogen than the cropped plot.

The water for irrigation at Belgaum contained appreciable quantities of nitrogen, especially in the form of nitrates amounting to 2·86 parts nitrogen per 100,000 parts of water. There were in all twenty-nine irrigations from the middle of December to the beginning of June 1934. The total quantity of nitrogen added through irrigation amounted to less than two milligrams per 100 grams of soil. This is perhaps the reason why there is a regular rise in nitrates in all the three plots from January onwards. The uncropped plots naturally show more nitrates than the cropped one. As soon as irrigation is stopped in the beginning of June the rains set in and the nitrates are washed away rapidly. Although the amount of nitrogen added through irrigation is sufficient to influence the increase in nitrates in the soil, it cannot account for the fluctuations in total nitrogen which are far greater than the nitrogen added by irrigation.

V. Nitrogen fluctuations in soil under heavy rainfall conditions

Experiments described in the previous sections furnished data for nitrogen fluctuations in soil under irrigation and with high rainfall followed by irrigation. A set of experiments was arranged at Karjat about sixty miles from Poona on the Bombay-Poona line, to study the nitrogen fluctuations in soils under heavy rainfall. On the Government Experimental Farm at Karjat, an area was divided into two equal plots adjacent to each other measuring 33 ft. × 33 ft. each. On one plot transplanted rice was grown and the other was left without a crop but received water and all other treatments just as the cropped plot. No plot received any manure. The usual practice is to plough the land as soon as the first monsoon rains are received. There is a second ploughing after about a fortnight. The land is puddled and planked in July just before transplanting rice seedlings. The crop is harvested in November.

The climatic data of Karjat are given in appendix C.

Usually washings from the upper fields are allowed to run in to the lower fields. This was not allowed in the experimental plots. The rain water was allowed to accumulate in beds, and excess allowed to run out.

The soil of the plots under experiment is one and a half feet deep lying over trap. The samples of soils were taken as previously described by randomisation and by preparing a composite sample from twelve spots from month to month. Fig. 4 will give an idea as to how the twelve spots for soil samples were distributed for the month of July 1933 only. Temperatures and mositures for both the plots were nearly the same, temperature varying within one °C. and moisture varying within one per cent. The total nitrogen figures are given in Table XVII.

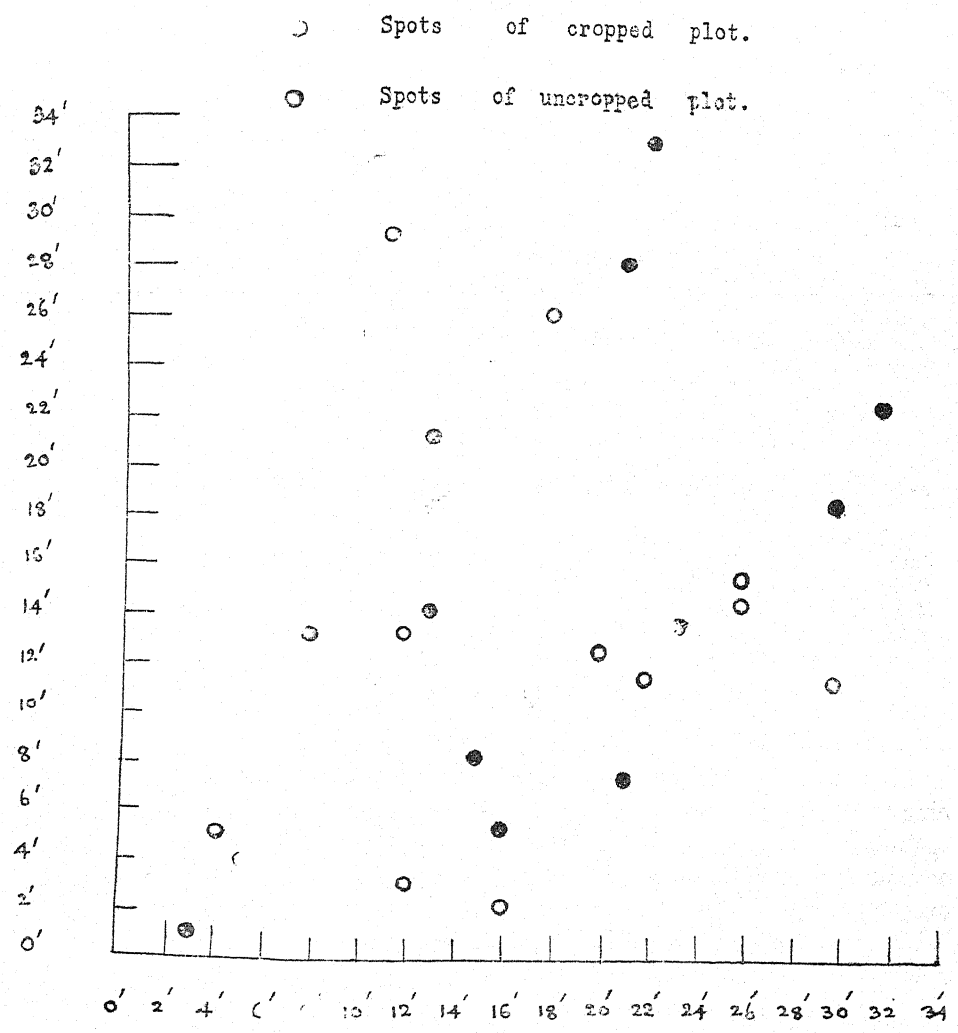

Fig. 4. Showing the distribution of the twelve spots of soil samples at Karjat for July 1933.

TABLE XVII

Temperature and moisture of the soil, pH values and total nitrogen in mgs. per 100 grams of oven-dry, fine soil

Time	Soil temperature	Soil moisture	pH value	Total nitrogen cropped plot	pH value	Total nitrogen uncropped plot
1933						
Second week of—	°C.	Per cent				
July . .	..	40·22	7·69	86·21	7·69	86·21
August .	..	40·22	7·61	86·00	7·21	85·90
September .	..	40·22	7·76	87·77	7·51	82·39
October .	30·2	36·80	7·78	83·78	7·53	82·58
November .	24·7	34·26	7·90	91·72	7·74	83·66
December .	26·7	16·71	7·97	88·60	7·73	84·50
1934						
January .	27·1	6·78	7·98	89·67	7·73	84·73
February .	32·0	6·66	8·03	86·11	7·71	82·46
March . .	39·3	6·65	7·91	86·05	7·70	86·29
April . .	44·6	6·40	7·90	86·92	7·68	83·94
May . .	40·4	6·94	7·85	82·04	7·64	84·85
June . .	26·0	33·15	7·95	87·67	7·56	85·15
July . .	..	40·22	7·64	88·28	7·64	81·19

In the uncropped plot the total nitrogen is practically the same from beginning to end except that there is a fall in the month of September 1933 and a fall again in July 1934 and a rise in March 1934. The other fluctuations are not much beyond working error. On the cropped plot, nitrogen is nearly steady during July, August and September, falls in October and rises distinctly in November. It is then nearly constant till January 1934 and comes down further and remains nearly at the same level throughout.

There is an important difference in the cropped and the uncropped plot. The cropped plot being almost always higher than the uncropped plot in total nitrogen. At the end of the cycle, the uncropped plot is lower in nitrogen than it started with, on account of the absence of crop and therefore for want of addition of organic matter. The cropped plot which receives organic matter in the form of roots and stubbles keeps its nitrogen contents at a higher level than the uncropped plot.

Here again it is seen that during the life time of the crop the nitrogen is higher in the cropped than in the uncropped as in the case of rice plots at Belgaum. It can therefore be stated that during the life time of the crop the cropped plot was higher because of the presence of rice crop and after the crop was harvested, the cropped plot had the advantage of the crop residues. The lowest figure of total nitrogen is 81·19 milligrams per 100 grams of oven-dry soil in July 1934 and the

highest is 86·29 milligrams per 100 grams of oven-dry soil in March 1934. The maximum fluctuation therefore is 6·27 per cent in the uncropped plot.

In the cropped plot the lowest total nitrogen is 82·04 milligrams per 100 grams of oven-dry soil in May 1934 and the highest is 91·72 milligrams per 100 grams of oven-dry soil in November 1933. The highest fluctuation therefor is of 11·8 per cent.

Both the cropped and the uncropped plots show but very little change in the pH values. Values are very slightly lower in the rains than in the other seasons. The range of the cropped plot is from 7·61 to 8·03 while that of the uncropped plot is from 7·21 to 7·74.

TABLE XVIII

Temperature and moisture of the soil and nitric nitrogen in mgs. per 100 grams of oven-dry, fine soil

Time	Soil temperature	Soil moisture	Nitric nitrogen in cropped plot	Nitric nitrogen in uncropped plot
1933 Second week of—	°C.	Per cent		
July	..	40·22	0·38	0·38
August	..	40·22	0·04	0·13
September	..	40·22	0·23	0·30
October	30·2	36·80	0·10	0·22
November	24·7	34·26	0·37	0·13
December	26·7	16·71	0·53	0·50
1934				
January	27·1	6·78	0·37	0·35
February	32·0	6·66	0·33	0·43
March	39·0	6·65	0·32	0·41
April	44·6	6·40	0·31	0·39
May	40·4	6·94	0·15	0·13
June	26·0	33·15	0·42	0·34
July	..	40·22	0·20	0·17

The nitric nitrogen is low during rains both in the cropped and the uncropped plots, but lower in the cropped than in the uncropped. From November onwards the nitrates rise and reach the highest point in December, slowly fall till May, rise again in June and begin to fall in July.

VI. Discussion of results

So far the results of each set of experiments have been discussed separately under each section. It is necessary now to consider the results of all the sets taken together in order that the conclusions arrived at may be of general application. The results obtained may be considered under the following heads :—

(1) Relation of organic matter in the soil to the total nitrogen, (2) fluctuation in total nitrogen, (3) conditions under which the highest point of total nitrogen is reached, (4) conditions under which the lowest level in total nitrogen is reached, (5) effect of manuring and cropping, (6) fluctuation in the nitric nitrogen, and (7) general.

(1) *Relation of organic matter to total nitrogen*

In finding out this relation it is better to consider only the uncropped and unmanured plots and compare the highest and lowest levels of total nitrogen with the proportion of organic matter in the soil. It is seen from the following figures that both the highest and the lowest levels of total nitrogen are higher in soils with larger proportion of organic matter.

Table XIX

Relation of organic matter to total nitrogen in soils

Plots	Organic matter	Per 100 grams of oven-dry, fine soil	
		Highest point of total nitrogen	Lowest point of total nitrogen
	Per cent	(Mgs.)	(Mgs.)
Poona *juar* (uncropped)	1·53	51·07	41·22
Karjat rice uncropped	1·81	86·29	81·19
Belgaum uncropped and unmanured (before manure was given to other plots)	4·44	236·47	197·69

At Belgaum organic matter as farmyard manure was added to plot B in December 1933 while plot C was not manured. In every other way both the plots were treated alike. Before the manure was added the total nitrogen of plot

C was not lower than that of plot B, but from January onwards plot B showed higher nitrogen than plot C and this might be put down as the effect of organic matter added (Fig. 5).

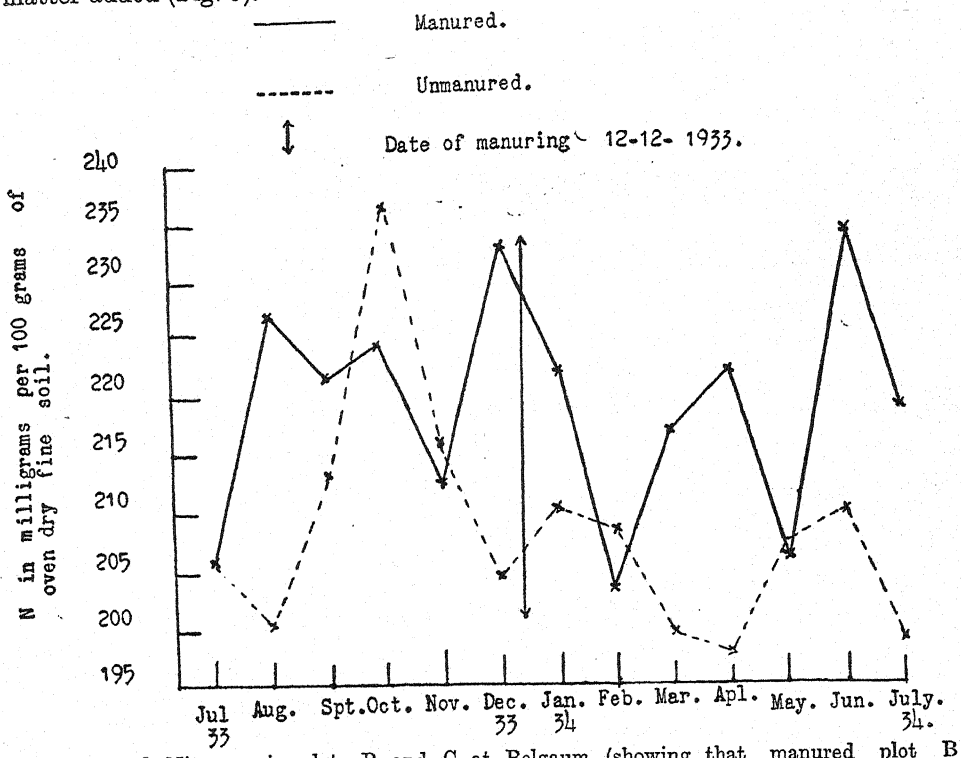

Fig. 5. Total Nitrogen in plots B and C at Belgaum (showing that manured plot B contains more total nitrogen than unmanured plot C after it is manured in the month of December).

TABLE XX

Total nitrogen in manured and unmanured plots

Plot	Condition	Per 100 grams of oven-dry, fine soil after manure was added	
		Highest point of total nitrogen	Lowest point of total nitrogen
C	Unmanured (after the other plot was manured in December)	210·26	197·69
B	Manured	234·42	203·63

(2) *Fluctuation in total nitrogen*

Fluctuation in total nitrogen does not seem to depend wholly on organic matter. It depends at least partially on the lime and the phosphoric acid contents of the soils [Sahasrabuddhe and Ghatikar, 1931]. Table XXI brings this out. It gives the organic matter, lime and phosphoric acid contents of the unmanured and uncropped soils with the maximum difference between the highest and the lowest proportions of total nitrogen and the percentage increase in each plot.

TABLE XXI

Soil constituents of unmanured and uncropped plots with the nitrogen fluctuations

Plot	Organic matter	Lime (CaO)	Phosphoric acid P_2O_5	Highest point of total nitrogen	Lowest point of total nitrogen	Per cent increase
	Per cent	Per cent	Per cent	(Mgs.)	(Mgs.)	Per cent
Poona (*juar*) uncropped .	1·53	6·17	0·054	51·07	41·22	23·1
Belgaum uncropped . .	4·44	1·05	0·36	236·47	197·69	19·6
Karjat uncropped	1·81	0·92	0·11	86·29	81·19	6·3

The larger the proportion of lime, the greater is the fluctuation in total nitrogen modified by the proportion of phosphoric acid. The actual quantity of total nitrogen may depend on the organic matter but the fluctuation appears to be affected by the proportion of lime and phosphoric acid contents [Sahasrabuddhe and Ghatikar, 1931]. The Karjat soil which contains only a small proportion of lime shows a very small fluctuation.

(3) *Conditions under which the total nitrogen reaches the highest point*

The highest point of total nitrogen is affected by many factors. Ordinarily the total nitrogen is high during the cold season from November to February. Although the minimum air temperatures in these months are low they are rarely so low as to interfere with the reactions in the soil. The maximum temperatures and the moistures in the soil taken together are favourable for the reactions taking place in the soil. In the cold season the minimum air temperatures may

go as low as 8°C. to 10°C. and there is, under these circumstances, sometimes, a fall in total nitrogen in the unirrigated plots as will be seen from the total nitrogen figures for January for the *juar* plots at Poona. During the cold season the mean maximum air temperatures varied from 27°C. to 34°C. and the mean minimum air temperatures varied from 8°C. to 24°C. The soil temperatures at 5 P. M. on the days of taking samples varied between 27°C. and 33°C. Sometimes another high figure for total nitrogen is obtained in June when the soils which get heated during summer are wetted by June rains.

TABLE XXII

Showing the plots and the months in which the highest or very nearly the highest figures for total nitrogen are obtained

Plots	Months
Poona *juar* cropped plot	December and September.
Poona *juar* uncropped plot	December and June.
Poona turmeric cropped plot	December and June.
Poona turmeric uncropped plot . . .	February and June.
Belgaum manured, cropped and irrigated plot .	November.
Belgaum manured, uncropped and irrigated plot	December and June.
Belgaum unmanured, uncropped and irrigated plot	October.
Karjat rice cropped plot	November.
Karjat rice uncropped plot	January and June.

(4) Conditions under which the total nitrogen reaches the minimum level

The minimum figures for total nitrogen do not fall in any particular month—the month changing with the treatment of the soil. The total nitrogen, however, is generally low in the hot weather when the moisture goes down. The lowest figures are in many cases in May because the moisture is very low. But sometimes very low figures of total nitrogen are also obtained in the rainy season, perhaps on account of the washing away by rains. Belgaum plots being irrigated for eight months form exceptions to the above statement.

TABLE XXIII

Showing the plots and the months in which the minimum level of total nitrogen is reached

Plots	Months
Poona *juar* cropped plot	July.
Poona *juar* uncropped plot	May.
Poona turmeric cropped plot	August.
Poona turmeric uncropped plot	May.
Karjat rice cropped plot	May.
Karjat rice uncropped plot	September.

For Belgaum plots, although the lowest figures are not in May, still the total nitrogen figures in the hot season are, on the whole, low.

(5) *The effect of cropping on the total nitrogen of soils*

The effect of crop on the total nitrogen is not the same with all the crops. The *juar* plots at Poona and the rice plots at Karjat show that the cropped plots contain more total nitrogen than the uncropped plots, both during the growing period of the crops and also after they are removed. At Belgaum during the growing period of rice, the cropped plot A contains more total nitrogen than the uncropped plots B and C. But when vegetables are planted after rice in Belgaum the same plot A shows less nitrogen in certain months than the uncropped plot B (treated exactly like A except for the crop). The turmeric plots at Poona show that when turmeric is growing on the land from July to March the cropped plot has less total nitrogen than the uncropped one. But as soon as the crop is removed, the cropped plot gets better than the uncropped plot. The question as to which crops help nitrogen recuperation during their growing period must form a problem by itself for an independent investigation (Figs. 6, 7, 8, and 9).

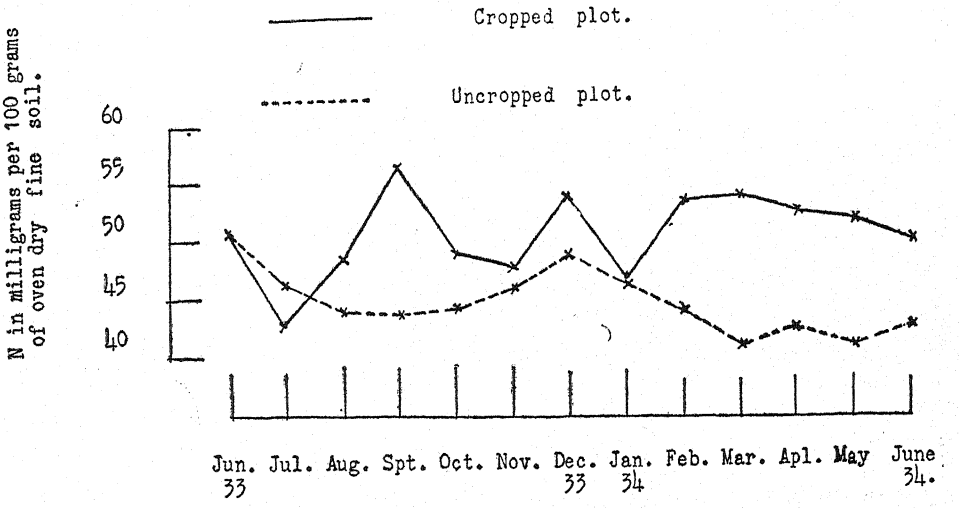

Fig. 6. Total nitrogen in *juar* plots, Poona (showing that the cropped plot always contains more total nitrogen than the uncropped plot).

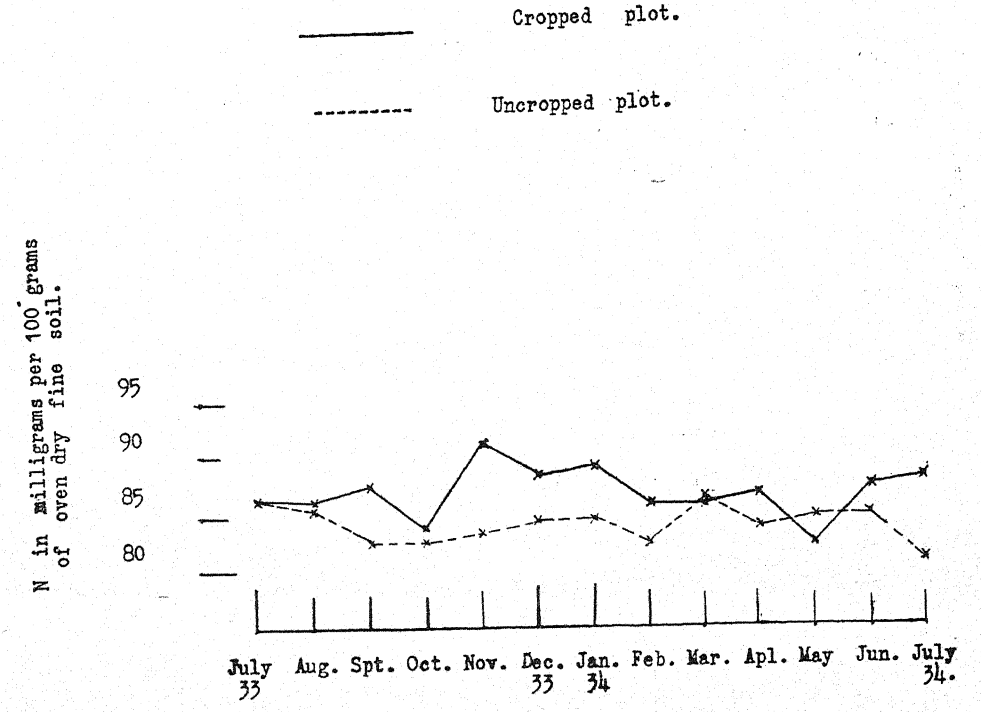

Fig. 7. Total nitrogen in rice plots at Karjat (showing that the cropped plot contains more total nitrogen than the uncropped plot).

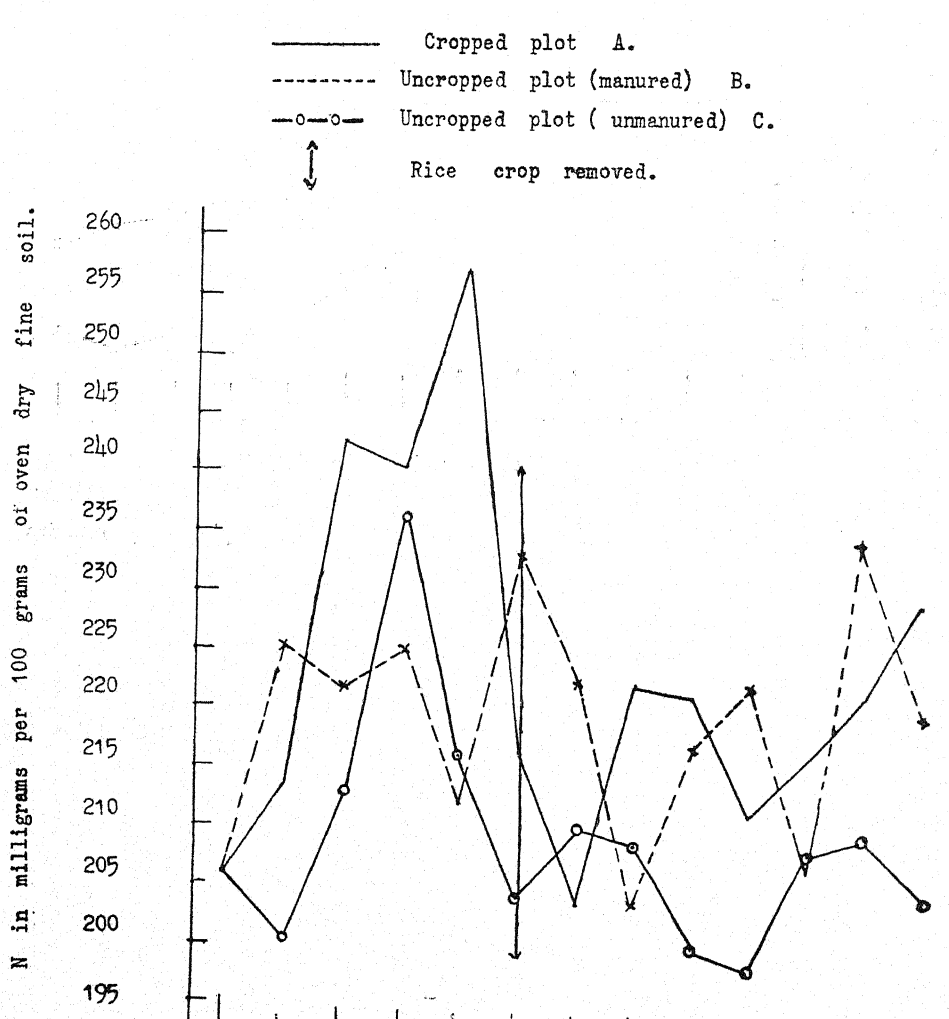

Fig. 8. Total nitrogen in plots A, B and C at Belgaum (showing that during the growing period of rice, the cropped plot A contains more total nitrogen than the uncropped plots B and C).

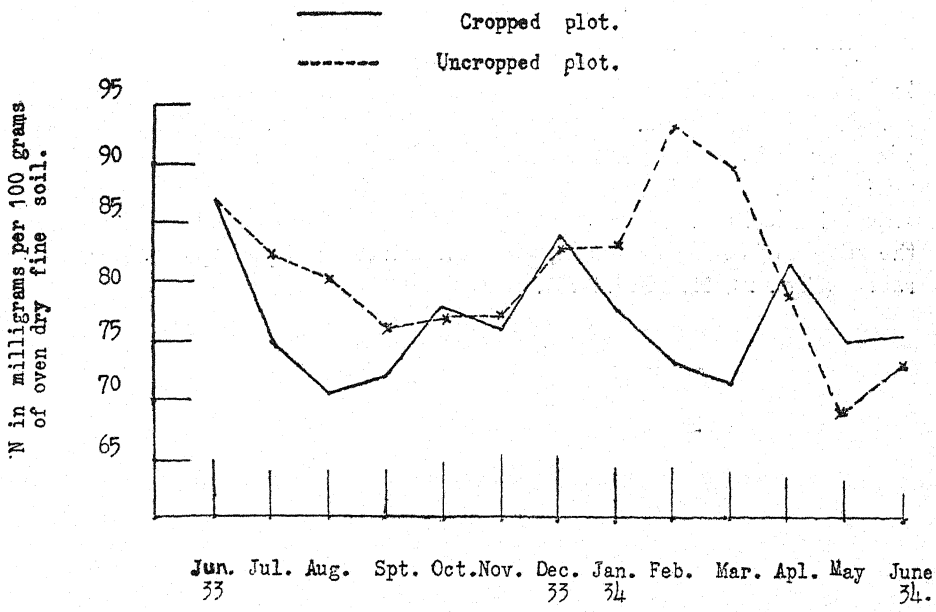

Fig. 9. Total nitrogen in turmeric plots, Poona (showing that the total nitrogen in uncropped plot is more than the cropped plot during the period while crop is on the land. After it is removed in March the total nitrogen in uncropped plot becomes less than the nitrogen in the cropped plot as in April 1934).

Although during the growing period of the crop, all crops do not show the same effect on the total nitrogen, yet after the crop is removed the residues of the crop left in the soil help the cropped plot to get more nitrogen than the uncropped ones in all the cases studied. Cropping therefore has a good effect. Manuring and cropping affect the total nitrogen in general and the highest point reached. The lowest figures however are affected only slightly and that too only at times as is seen from Table XXIV given below.

TABLE XXIV

Comparison of minimum total nitrogens in the cropped and the uncropped plots (Mgs. per 100 grms. of oven-dry soil)

Crop and place	Cropped plots	Uncropped plots
Juar at Poona	43·38	41·22
Turmeric at Poona	70·59	69·11
Rice at Belgaum	203·51	203·63
Rice at Karjat	82·04	82·46

(6) *Fluctuations in nitric nitrogen*

With the exception of the Belgaum plots, which may be considered separately, it is observed that all the plots, both cropped and uncropped, show high proportion of nitric nitrogen during the cold season from November to February when the moisture and temperature conditions taken together are favourable for nitrification. Sometimes nitrification is active in June when the soils get wetted after being heated in the hot season. The lowest figures of nitric nitrogen are in the middle of the rainy season, since the nitrates are perhaps washed away by rains (Figs. 10, 11, 12 and 13).

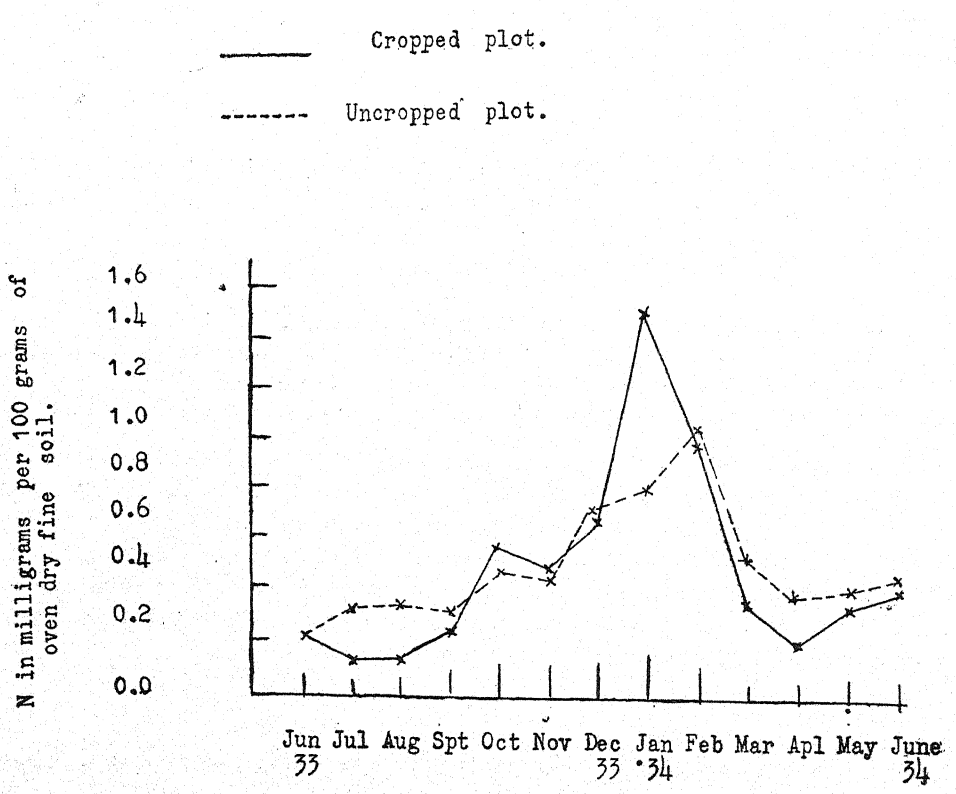

Fig. 10. Showing the nitric nitrogen in cropped and uncropped plots of *juar* at Poona.

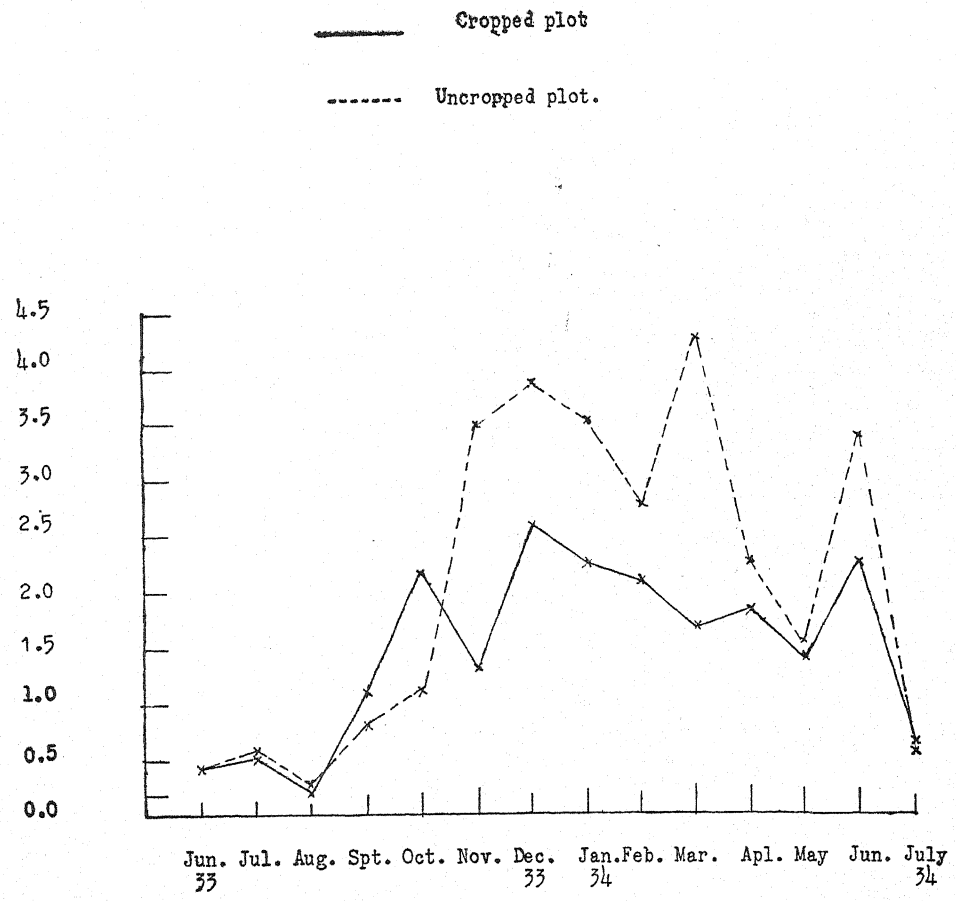

Fig. 11. Showing the nitric nitrogen in cropped and uncropped plots of turmeric at Poona.

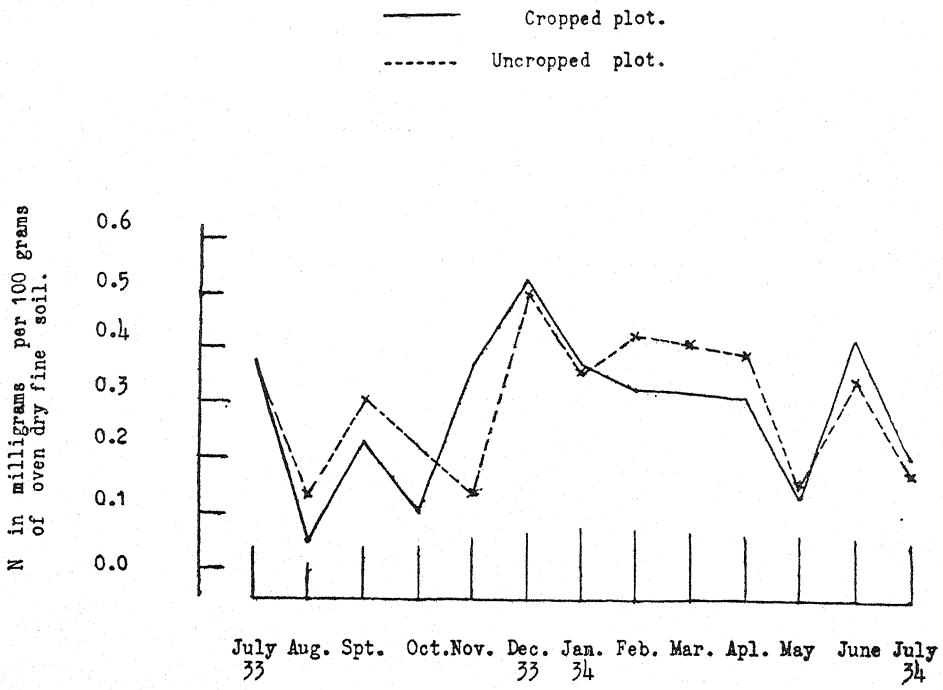

Fig. 12. Showing the nitric nitrogen in cropped and uncropped plots at Karjat.

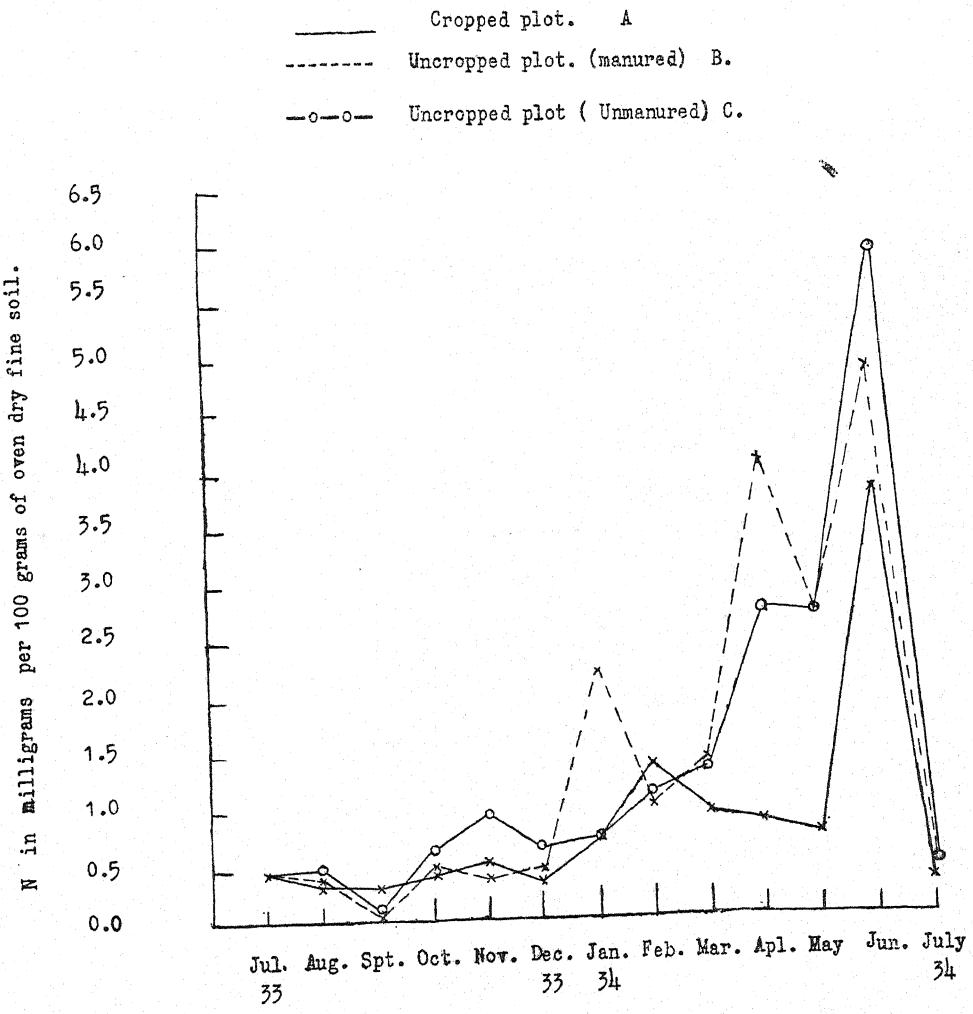

Fig. 13. Showing the nitric nitrogen in plots A, B and C, at Belgaum.

TABLE XXV

Showing the plots and the months showing minimum and maximum nitric nitrogen

Plots and places	Months of minimum nitric nitrogen	Months of maximum nitric nitrogen
Poona *juar* cropped plot	July and August	January
Poona *juar* uncropped plot	July . .	February
Poona turmeric cropped plot	August . .	December
Poona turmeric uncropped plot	August . .	December and March
Karjat rice cropped plot	August . .	December
Karjat rice uncropped plot . . .	August and May	December

Generally the nitric nitrogen is higher in the uncropped plots than in the cropped plots.

In the Belgaum plots the nitric nitrogen is low during the rains but the highest mark is not reached in the cold season but goes on increasing till June. This is so because the plots were irrigated from November to June with water which contained nitrates. The difference due to the crop is clear at Belgaum because plots B and C which were not cropped show more nitric nitrogen than plot A which was cropped throughout the period from November to June.

(7) *General*

It is contended by some that the soil heterogeneity is so much that no accurate estimate of the various changes in nitrogen in soil can be obtained by carrying out experiments in the field. Such investigation must therefore be carried out under controlled conditions of the laboratory.

It may be made clear here that the study of the nitrogen recuperation was first done in the laboratory, under controlled conditions [Sahasrabuddhe and Daji, 1925 and Sahasrabuddhe and Ghatikar, 1931]. After definite results were obtained there, field experiments were undertaken to see what actually takes place under the usual conditions of cultivation and climatic changes.

In order to get an exact idea about the periodic fluctuations in the nitrogen of the soil it is necessary to study the changes for a cycle of twelve months and this was done in the investigation under consideration. All possible precautions

were taken in collecting samples. The sampling error in experiments is small and the differences in the nitrogen contents of the soil cannot be put down to error of any kind. The plots chosen represent distinct types of cultivations. The results obtained are not erratic but show some regular tendencies in the changes as discussed above. The changes, therefore, must be taken as indications of what actually happens.

VII. Conclusions

1. The nitrogen of the soil, under the conditions of the Bombay-Deccan, is not a constant quantity. It fluctuates from month to month according to moistures and temperatures of the soil, which depend in their turn on the climatic conditions, thus confirming the results published by Sahasrabuddhe and Kanitkar [1932]. There is an annual cycle of these fluctuations, indicating regular loss and recuperation of nitrogen by the soils. This holds good not only for the *rabi* lands as shown by Sahasrabuddhe and Kanitkar [1932] but also for *kharif* dry lands, heavy rainfall lands and for lands under irrigation.

2. In the *kharif*, irrigated and heavy rainfall soils the highest point of total nitrogen is reached in cold season and some times in June also. The lowest level is reached in July or August, *i.e.*, in the middle of the rainy season or in May when the moisture is very low.

3. The larger the quantity of organic matter in the soil, the greater is the proportion of nitrogen in the soils.

4. Addition of organic matter as manure or in the form of roots and leaves of crops helps in fixing nitrogen by the soils.

5. The fluctuations in total nitrogen depend, at least partially, on the proportion of lime and phosphoric acid contents of soils.

6. During the growing period of a crop the cropped cultivated land is better than the uncropped cultivated land with certain crops, as rice and *kharif juar* but the reverse is the case with certain other crops, as irrigated vegetables and turmeric. This question however requires further investigation.

7. In all the cases studied it was observed that after the crop was removed the cropped soils were better in nitrogen than the uncropped ones.

8. When a soil is cultivated it is better to crop it than to leave it uncropped.

9. Nitric nitrogen is higher in cold season which extends from November to February and in June at the beginning of the rains.

10. During the life of the crop, the cropped plots contain less nitric nitrogen than the uncropped plots.

Acknowledgments

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Appendix A

The climatic data noted on the Poona Agricultural College Farm for the year 1933-34.

Month and year	Rainfall during the month	Mean temperature of the month and of the week of sample taken		Mean humidity
		Maxima	Minima	
	(Inches)	(°C.)	(°C.)	(Per cent)
1933				
May	5·98	36·66	21·94	78·8
June	5·43	33·47	21·66	83·2
Week of sample	3·23	27·22	20·55	93·0
July	5·89	29·30	21·66	88·4
Week of sample	4·62	27·22	21·11	94·9
August	7·24	27·98	20·83	89·8
Week of sample	0·29	29·44	20·55	86·5
September	8·29	29·56	20·55	88·22
Week of sample	0·85	31·11	19·44	85·50
October	1·51	31·02	18·46	85·6
Week of sample	..	30·55	13·89	84·7
November	0·21	30·96	13·68	77·6
Week of sample	..	31·11	14·72	79·3
December	1·00	25·97	9·93	82·4
Week of sample	..	28·89	8·33	83·8
1934				
January	..	29·58	8·61	80·09
Week of sample	..	30·55	10·55	78·1
February	..	33·68	9·44	71·6
Week of sample	..	33·89	11·11	65·5
March	..	36·36	13·59	65·4
Week of sample	..	40·00	16·90	62·7
April	..	40·05	22·29	65·1
Week of sample	..	39·66	18·33	59·3
May	0·28	39·08	21·11	58·0
Week of sample	..	38·33	22·22	59·22
July	3·90	32·56	21·80	78·1
Week of sample	1·05	29·16	21·67	84·4

Appendix B

The climatic data noted at Belgaum for the year 1933-34

Month and year	Rainfall of the month and of the week in which the sample was taken	Mean temperature of the month and of the week of the sample taken		Mean humidity
		Maxima	Minima	
	(Inches)	(°C.)	(°C.)	(Per cent)
1933				
May	5·1	32·1	19·8	76·7
June	9·3	25·6	19·9	89·7
July	16·50	25·14	19·74	91·5
Week of sample	0·10	26·1	20·0	89·6
August	12·4	25·25	19·41	91·8
Week of sample	8·90	22·6	19·7	94·1
September	6·0	26·71	17·32	90·14
Week of sample	1·90	27·5	13·6	93·9
October	8·8	27·92	13·4	80·88
Week of sample	4·0	29·4	18·6	84·0
November	3·20	28·86	16·65	62·2
Week of sample	..	29·4	14·7	47·5
December	0·30	27·88	13·19	61·6
Week of sample	..	27·2	11·9	52·4
1934				
January	..	28·4	14·44	68·15
Week of sample	..	26·9	13·9	74·1
February	..	31·99	15·86	51·0
Week of sample	..	29·7	16·7	53·0
March	..	34·12	16·56	48·7
Week of sample	..	32·2	14·7	42·1
April	0·7	35·6	19·45	70·0
Week of sample	..	36·7	19·7	70·1
May	0·6	34·20	19·78	67·50
Week of sample	0·6	36·7	20·0	66·6
June	9·90	27·66	20·27	86·9
Week of sample	3·20	27·6	20·6	89·7
July	18·8	24·30	19·78	94·20
Week of sample	11·8	23·33	19·6	94·0

Appendix C

The climatic data noted at Karjat for the year 1933-34

Month and year	Rainfall of the month and of the week in which the sample was taken	Mean temperature of the month and of the week of the sample taken		Mean humidity
		Maxima	Minima	
	(Inches)	(°C.)	(°C.)	(Per cent)
1933				
May	5·10	35·83	24·20	71·60
June	27·64	30·53	22·50	84·20
July	44·97	28·24	23·90	86·90
Week of sample	6·56	28·89	23·89	82·70
August	45·53	27·60	22·90	84·90
Week of sample	2·82	28·89	23·59	89·60
September	24·86	29·00	23·60	89·20
Week of sample	6·31	27·78	23·30	89·90
October	4·49	31·80	21·70	89·00
Week of sample	0·13	30·55	21·10	85·30
November	..	32·80	16·50	81·10
Week of sample	..	32·22	17·78	83·00
December	1·53	30·76	12·72	79·50
Week of sample	1·53	29·44	16·87	77·70
1934				
January	..	30·27	11·25	90·10
Week of sample	..	30·55	7·22	96·00
February	..	34·44	12·60	74·10
Week of sample	..	33·89	12·80	76·30
March	..	36·11	15·30	68·00
Week of sample	..	37·80	17·80	62·60
April	..	45·90	21.85	73·20
Week of sample	..	37·22	22·20	71·30
May	..	38·05	23·19	74·80
Week of sample	..	38·33	23·30	69·80
June	33·29	30·99	22·80	80·90
Week of sample	3·19	31·11	17·20	83·30
July	36·34	27·80	23·32	87·20
Week of sample	7·16	28·10	23·50	85·00

NITROGEN BALANCE IN BLACK COTTON SOILS IN THE MALWA PLATEAU, II

CHANGES DURING THE HOT WEATHER

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INTRODUCTION

Very low yields of seed cotton—about 150 to 500 lbs. an acre—are obtained on the black cotton soils of Malwa in contrast with the higher production—300 to 1,500 lbs. an acre—from Punjab soils. In most of the high-yielding, arid areas cotton is sown in the hot weather much before the rains. When similarly sown in advance of the rains in Malwa local varieties also yielded equally high. [Kubersingh and Wad, 1934, unpublished]. Similar results from early sown crops have been reported from other parts of India [Burt, 1919; Hilson, 1928; Main, 1908]. Being very well convinced of the depressing influence on yield if sowings are delayed after the rains break, the Malwa cultivator takes good care to finish his sowings rapidly on the beginning of the monsoon. Other crops behave similarly; maize is preferably sown in the middle of May on garden lands in Malwa. In an experiment at Indore in 1933 the maize varieties, Fitzroy and Imperial Yellow Dent gave acre-yields of 2479 and 2120 lbs. for the hot-weather-sown crops and 479 and 256 lbs. for those sown on rains.

Better nitrogen nutrition appeared at least partly responsible for these increments in yields. Before any more efficient system of cultivation could be devised to secure better yields of cotton it seemed essential to secure an accurate estimate of the equilibria and intensities of nitrogen, nitrified or in another form, that may exist in the black cotton soils during the growth periods of an irrigated,

hot-weather-sown cotton crop. Direct exposure of the soil surface—wet or dry—to sunlight, and protection of the lower layers from light and to some extent from the effect of wind, are the main features in the environment of a hot-weather crop in its early stages. Under conditions very closely approximating to those in the field, determinations were made of the influence of direct sunlight and temperatures on the nitrification as well as the conservation of nitrogen, both in dry and moist soils, with and without manures. The theory has recently been put forward that nitrification in the tropics occurs mainly through photo-chemical and not biological agencies [Dhar and Rao, 1931]. Sarkaria and Fazal-ud-Din [1934], however, suggest the need of further confirmation before finally accepting this view. The work to be described gives further information on the subject.

Materials and methods

During the summer of 1933 an attempt was made to estimate the effect of the factors given below on the nitrification and conservation of nitrogen in the surface two inches of soil :—

(1) Soil moisture.

(2) Exposure to direct sunlight.

(3) Atmospheric humidity.

(4) Inorganic and organic manures applied at normal field rates.

The parts played by the biological and the recently-suggested photo-chemical agencies during nitrification [Dhar, Bhattacharya and Biswas, 1933; Tandon and Dhar, 1934] were also determined.

The technique used was essentially that described in Part I of this series [Wad and Panse, 1933]. Soil, evenly graded by passing through a 2-mm. sieve, was uniformly filled in galvanised iron trays 12 in. $\times$ 6 in. and 2 in. deep. The soils used were either unmanured or were mixed thoroughly with compost or ammonium sulphate equivalent to a rate of 17 lbs. of nitrogen per acre/two inches. Half the number of trays were kept in the open in an uncropped field on suitable supports, eighteen inches above ground level and exposed to direct sunlight, side by side with an equal number kept screened from the sun by a corrugated iron sheet roof sloping towards the North and by removable screens to the East or West as needed. Half the number of trays in each set contained dry soil. Sufficient water was added to raise the water content to thirty per cent. The actual daily fluctuations in moisture content ranged between the minima and maxima of fifteen and thirty per cent respectively in covered trays and ten and thirty per cent in those exposed, all losses being replaced daily. The hygroscopic moisture in the original

soil was 9·3 per cent and its saturation capacity was 60·0 per cent. Both unmanured and manured soils were included in each group and the position of each tray within a group was interchanged daily at random. The arrangement may be represented thus :

Sun		*Shade*	
Watered	Unwatered	Watered	Unwatered

Temperatures in the vicinity of each group were recorded daily at 11 A.M. and 3 P.M. (Figs. 1, *a* and *b*). The temperatures in the shade were lower on an average by 5° F. than those in the sun during the hottest period of the day in the warmer spells, the difference being even less on cooler days. The Campbell-Stokes sunshine recorder—universal pattern—was used to record the intensities and duration of sunshine. According to the breadth of markings on the paper the total period of daylight was divided into three portions representing low, medium and high light intensities.

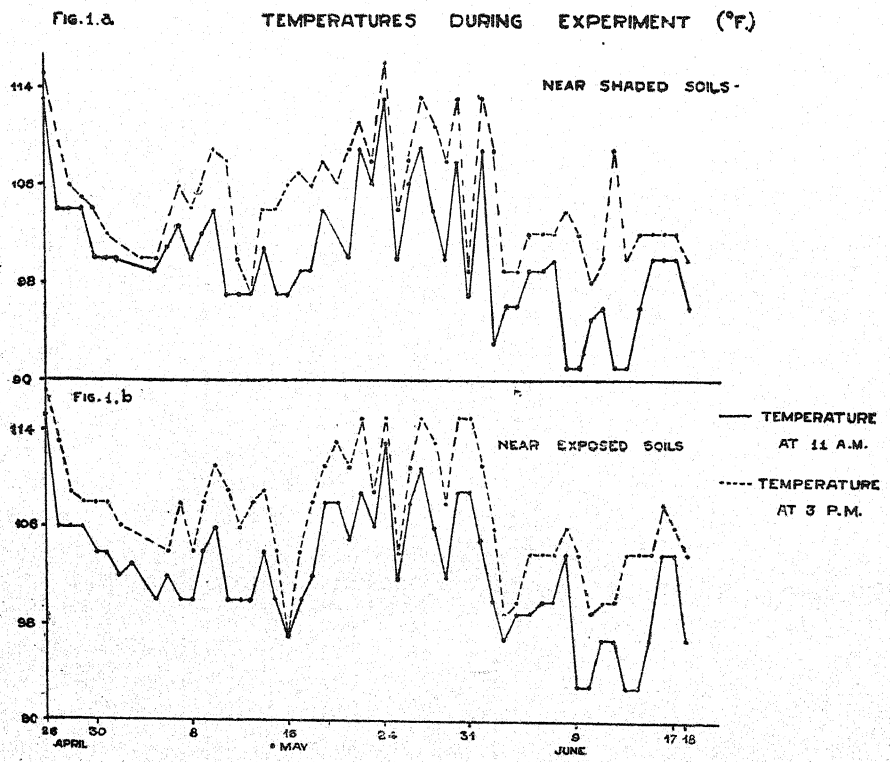

Fig. 1. Temperatures during experiment (°F.)

TABLE I

Hours of sunshine during the period of nitrification

Exposure	Total hours of			
	high intensity	medium intensity	low intensity	Total
19th April—20th May	63	116	27	206
21st May—19th June	64	92	39	195
Total	127	208	66	401

In order to estimate the extent of the photo-chemical activity, several 100-grm. lots of soil, dry as well as wetted, with and without manures as in the main experiment, were sterilised in culture flasks, Roux's pattern 8 in. × 5 in. × 2 in. plugged with cotton wool. The dry soils were sterilised at 150° C. for twelve hours and the wetted soils at 120° C. in an autoclave for eight hours. An equal number of flasks of each kind were kept side by side both in light and in darkness at air temperatures, care being taken to interchange the positions of the flasks in each set to ensure uniformity throughout the experiment. The results obtained were again confirmed by another trial with unmanured soil.

The unsterilised sets of the main experiment were started on 19th April and continued up to 19th June 1933. Nitrates were determined by the phenol-di-sulphonic-acid method and total nitrogen by the modified official Kjeldahl method after thirty-one and sixty-one days in the unsterilised set and after thirty-two days in the sterile set. Any chance infections of the sterilised sets during the period of experiment were traced by plating out each sample, and the nitrogen changes in such infected samples were considered separately. All results were statistically evaluated by Fishers' 't'-test, P less than 0·05 being taken as the standard for significance. The results are expressed in terms of means of all determinations along with the values of 't' for the pairs compared. The value of 't' to make each comparison valid on a basis of 19 : 1 is also given for the number of degrees of freedom available. All comparisons made in the text are invariably based on differences which are either significant or insignificant statistically, these terms have therefore been omitted to avoid repetition.

RESULTS AND DISCUSSION

The extent of photo-chemical nitrification in the absence of micro-organisms

The experiments with sterile soils failed to show any significant increases under the influence of sunlight, both in nitrates and total nitrogen (Table II *a*). Wherever subsequent chance infection was found, significant increases occurred directly proportional to its degree (Table II *b*) as shown by the number of colonies on plating.

TABLE II

Nitrogen changes in sterile and chance-infected soils in sunlight and shade (Mgms. of nitrogen per 100 grms. of oven-dry soil)

(*a*) *Sterile samples*

No. of test	Form of nitrogen	Sun	Shade	Sun *vs.* shade *t*
19th April to 19th May .	*With water*			
1.				
a . .	Nitrate nitrogen .	2·38	1·81	1·74
b . . .	Total nitrogen . .	62·41	62·14	0·45
16th Oct. to 16th Nov. .	*With water*			
2.				
a . . .	Nitrate nitrogen .	3·59	2·84	0·91
b . . .	Total nitrogen . .	65·56	65·72	0·77
3.	*Without water*			
a . . .	Nitrate nitrogen .	3·08	2·87	0·51
b . . .	Total nitrogen . .	67·38	66·64	0·33

(*b*) *Infected samples*

No. of test	Form of nitrogen	Infection		Much infected *vs.* less infected
		Much	Less	
19th April to 19th May .				
4.				
a . . .	Nitrate nitrogen .	5·02	3·22	5·40
b . . .	Total nitrogen . .	69·33	64·26	3·43

NOTE :—n=34 for test No. 1 (a & b).
10 for Nos. 2 (a & b) and 3 (a & b).
9 for No. 4a.
8 for No. 4b.
t= 2·042 or more for test No. 1.
2·228 or more for Nos. 2 and 3.
2·268 or more for No. 4a.
2·306 or more for No. 4b.

In this and subsequent tables a comparison is significant only when the value of "t" opposite that comparison is greater than the required "t" value for them specified at the foot of the table. By a significant comparison is here meant one in which the odds in favour of its being correct are at least nineteen to one.

Incidentally the results emphasize the extreme importance in experiments of this type of making sure, by actual proof, of the absence of subsequent infections, before inferences are drawn. Once infection occurs, organisms multiply very rapidly under favourable conditions. The absolute need of a biological agency to produce nitrogen changes, as well as the complete inability of photo-chemical action in producing such changes are simultaneously proved by these results. At any rate this is true of soils unmanured, treated with ammonium sulphate or with humic compost in doses used in farming practice which are evidently capable of providing the whole range of nitrogenous raw materials susceptible to photo-oxidation.

Temperatures as high as 115° F. did not prevent the existence of micro-organic activity both in the infected samples and the two-inch layers of the unsterilised soils used in the main test. The activity of azotobacter at 46° C. (115°F.) has been known [Green, 1932]. In arid regions even in the hottest season the initial temperatures of over 50° C. continuously existing in moist compost heaps for days together do not seem ever to have prevented the subsequent establishment of biological activities including that of nitrification. The infection

evidently has escaped the annihilation to be expected from the death-point determinations made inside a laboratory. The absolute uniformity of conditions so sure in the technique of laboratories may not exist within the large colloidal mass of soils. Otherwise the existence in and isolation of organisms from field or garden soils in extreme climates would have been a difficult task. It is essential to possess correct information regarding the nature of the factors really controlling nitrification in tropical soils, because an efficient adjustment of agricultural practice must be able fully to utilise those factors. The suggested existence of formidable obstacles to biological nitrification [Tandon and Dhar, 1934] can perhaps still be reconciled with the display of its predominance in the tests here described. It is not even necessary to assume the existence of unknown organisms or the survival and activity of familiar species under very drastic conditions. The soil processes are dynamic. The seat of active nitrification need not always coincide with the soil zones having maximum nitrate accumulation, nor are such accumulations necessarily derived from intense activity; they may result from a persistent but weak activity in a deeper zone.

The rapid decrease in soil temperatures with depth, as indicated at Lyallpur by a fall from 50° C. to 37° C. within 30 cm. only (as mentioned by Tandon and Dhar) makes clear the existence of more favourable temperatures a little lower down. At such depths the supply of oxygen is ample but even if it were not, it must be remembered that the aerobic nodule organisms can retain at least twenty-five per cent of their activity with a concentration of oxygen even as low as 0·5 per cent [Barthel, 1930]. The zone of active nitrification hence may quite easily shift, perhaps with changed intensities, to different depths from season to season according to the nature of the soil. The accumulation at the soil surface of salts, including nitrates, in arid climates [Gardner, Kezer and Ward, 1934] or during dry seasons, is a consequence. Under such conditions a wide range exists for the transport of salts to the surface (through the upward movement of moisture from the subsoil to be accumulated there on drying). The coincidence of such accumulations with hot and dry seasons is hence as easy to understand as their downward movement by leaching in wet weather. Even in extremely rigorous climates biological nitrification can thus be carried on safely beyond the reach of light without any danger of the organisms being poisoned by formaldehyde liable to be produced photo-chemically.

Instead of being directly dependent upon the production of nitrates, the physical improvement of soil by exposure during hot seasons may be due simply to the increased stability of the structure of its aggregates—a direct result of the irreversibility produced in the cementing material of the humus-clay complex when the former is coagulated by calcium [Sokolovsky, 1930]. Such a granular structure controls the efficiency of the water, air and biological regime in the soil. The high exchange capacity [McGeorge, 1930] of the argillo-humus fraction when suitably reinforced with calcium enables the soil to resist better the inevitable

deterioration of its structure—due to the disturbance of the equilibrium between soil-colloids and the absorbed calcium [Sokolovsky, 1930] which must be caused by crop-growth and biological activities like nitrification. In the absence of stable soil structure even "nitre spots" may be barren. The phenomenon of nitrification even in the tropics can thus, after all, still be the same as elsewhere.

NITRIFICATION

(*a*) *Watered soils*

Nitrogen changes in watered soils during periods of thirty-one and sixty-one days are given in Tables III and IV (*a* and *b*). Table V gives the accumulations and percentages of nitrified nitrogen in sunlight as well as in shade.

TABLE III

Nitrogen changes in watered soils during 31 days

(*a*) *On oven-dry basis*

Treatment	Nitrate accumulation (Mgms. N per 100 grms. of soil)		Nitrified N on total (Average of percentages)		Total nitrogen (Mgms. N. per 100 grms. of soil)			Average of per cent losses after 31 days	
					At start	After 31 days			
	Sun	Shade	Sun	Shade		Sun	Shade	Sun	Shade
Compost (A) . . .	5·81	4·45	7·11	5·45	81·74	76·79	74·32	5·94	9·06
Amm. Sulph. (B) . .	5·50	4·55	6·92	5·72	79·53	77·76	75·54	3·07	5·20
Control (C) . . .	5·48	3·92	7·88	5·55	70·68	58·68	59·99	16·98	15·24

(*b*) *Comparisons* (*values of* t)

Treatment	Sun *vs.* shade			Comparisons	Sun			Shade		
	Nitrate accumulation	Per cent nitrified N	Nitrogen losses		Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Nitrate accumulation	Per cent nitrified N	Nitrogen losses
A	5·24	5·19	2·31	A *vs.* B	1·22	0·59	1·97	0·48	1·02	2·22
B	4·52	4·53	1·53	A *vs.* C	0·90	1·62	8·21	2·02	0·30	3·45
C	4·40	4·80	1·30	*B vs.* C	0·06	2·18	12·20	3·07	0·51	6·04

NOTE :—$n=14$ throughout, $\therefore t=2·145$.

TABLE IV

Nitrogen changes in watered soils during 61 days

(a) On oven-dry basis

Treatment	Nitrate accumulation (Mgms. N per 100 grms. of soil)		Nitrified N on total (Average of percentages)		Total nitrogen (Mgms. N per 100 grms. of soil)			Average per cent losses after 61 days	
					At start	After 61 days			
	Sun	Shade	Sun	Shade		Sun	Shade	Sun	Shade
Compost (A) . . .	7·15	5·61	8·75	6·86	81·74	72·14	75·93	10·83	7·17
Amm. Sulph. (B) . .	6·56	5·24	8·25	6·59	79·53	74·22	73·48	6·66	8·06
Control (C) . . .	4·56	4·29	6·45	5·92	70·68	57·38	58·16	18·81	18·07

(b) Comparisons (values of t)

Treatment	Sun *vs.* shade			Comparisons	Sun			Shade			1st *vs.* 2nd period		
	Nitrate accumulation	Per cent nitrified N	Nitrogen losses		Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Treatment	Sun	Shade
A	9·34	9·45	2·72	*A vs.* B.	3·69	2·56	3·03	2·85	1·54	0·75	A	3·02	1·34
B	11·00	10·38	1·14	A *vs.* C.	14·39	9·79	6·03	9·43	2·99	7·81	B	3·02	1·97
C	1·68	1·54	0·54	B *vs.* C.	13·34	9·00	12·06	7·31	2·06	6·37	C	1·98	1·66

Note.—$n = 14$ throughout $\therefore t = 2{\cdot}145$.

TABLE V

Percentage changes in and net values of nitrate accumulations and intensities of nitrification in each period in watered soils

Treatment	Accumulations—Mgms. N per 100 grms. of soil								Intensities—percentages of nitrate N on total nitrogen							
	Percent increase in sun over shade		Sun			Shade			Percent increase in sun over shade		Sun			Shade		
	During		During		Per cent decrease during 2nd period	During		Per cent decrease during 2nd period	During		During		per cent. decrease during 2nd period	During		Per cent decrease during 2nd period
	1st period	2nd period	1st period	2nd period		1st period	2nd period		1st period	2nd period	1st period	2nd period		1st period	2nd period	
Compost	30·56	15·52	5·81	1·34	76·60	4·45	1·16	73·92	30·46	16·31	7·11	1·64	76·93	5·45	1·41	74·13
Amm. Sulph.	20·87	65·12	5·50	1·06	80·72	4·55	0·69	84·84	20·98	52·84	6·92	1·33	80·60	5·72	0·87	84·78
Control.	39·79	348·60	5·48	Loss 0·92	116·80	3·92	0·37	90·55	41·99	486·50	7·88	Loss 1·43	118·10	5·55	0·37	93·33

The favourable influence of exposure to sunlight was always visible with th only exception of untreated soils during the second period. The untreated soils received the greatest stimulus by light during the first period inspite of the absence of added easily oxidisable nitrogen. Ammonium sulphate though readily oxidisable fell behind compost which topped the list. During the second period there was a general drop in activity resulting in actual depression in untreated soils. It may be pointed out that there was no dearth of total nitrogen, though some was lost, but these losses were significant only with treatments in sunlight. Also treated soils suffered from depression in activity less than controls, while compost resisted it better than ammonium sulphate.

Evidently something other than sunlight or the micro-organisms or the nitrifiable material appears to be the controlling factor. Compost has exploited this to the best advantage, thus indicating that organic matter of a certain type was the decisive element. The nitrifying activity is lowered when this organic matter is not replenished. It may be that the organic carbon in the argillo-humus fraction is adversely affected by the very process of nitrification and the structure of the soil aggregates deteriorates.

The most efficient recovery of nitrogen in nitrified form from soils seems to be possible only when the process starts at a high intensity before the unfavourable phase gets the upper hand. Otherwise as time goes on the soil efficiency is reduced, probably due to its deterioration and the chances of recovery become smaller and smaller perhaps because the waste of nitrogen is increased by its being used up in other reactions going on side by side. This is clearly illustrated by the greater depression of activity under shade as time advances, in comparison with that in sunlight which stimulates the nitrification. This stimulus brings about the deterioration phase quickly in untreated soil but ammonium sulphate and, to a greater extent, compost retard its appearance and much more in light than in shade.

This point and the similarity in behaviour of ammonium sulphate and compost—two substances widely differing in their properties—requires careful investigation. The oxidation of ammonium sulphate in soils does not seem to be an independent chemical reaction. On the assumption of the intervention of the agency of micro-organisms there seems to be an intimate association of oxidation of ammonium sulphate with a source of energy available in close contact with it. The similarity referred to suggests humus-carbon as the source of energy. The quicker fall in activity of untreated soils than of those ammonium sulphate-treated which had no additions of organic matter and yet had to deal with larger quantities of nitrogen indicates that ammonium sulphate utilises soil humus more efficiently unless it provides itself with some other source of energy. A closer study of the mechanism by which ammonium sulphate is oxidised in soils is likely to give information useful for practical soil management.

(b) Unwatered soils

Results are given in Tables VI and VII, (*a* and *b*) and VIII.

TABLE VI

Nitrogen changes in unwatered soils during 31 days

(a) On oven-dry basis

Treatment	Nitrate accumulation (Mgms. N per 100 grms. of soil)		Nitrified N on total (Average of percentages)		Total nitrogen (Mgms. N per 100 grms. of soil)			Average of per cent losses after 31 days	
					At start	After 31 days			
	Sun	Shade	Sun	Shade		Sun	Shade	Sun	Shade
Compost (A) . . .	3·20	3·40	3·91	4·27	81·74	74·55	75·53	8·80	7·41
Amm. Sulph. (B) .	3·13	3·18	3·75	3·99	79·53	72·80	74·94	8·83	6·41
Control (C) . . .	2·80	3·08	4·50	4·36	70·68	62·64	60·61	11·15	10·39

(b) Comparisons (values of t)

Treatment	Sun *vs.* shade			Sun				Shade		
	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Comparisons	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Nitrate accumulation	Per cent nitrified N	Nitrogen losses
A	1.34	2.00	0.78	A *vs.* B	2.80	1.46	0.01	1.00	1.12	0.61
B	0.16	1.17	1.38	A *vs.* C	6.15	2.41	1.43	1.83	0.05	1.73
C	2.80	0.55	0.48	B *vs.* C	5.50	3.26	1.43	0.56	1.57	1.79

Note.—$n=14$ throughout. $\therefore t=2.145$

TABLE VII

Nitrogen changes in unwatered soils during 61 days

(a) On oven-dry basis

Treatment	Nitrate accumulation (mgms. N per 100 grms. of soil)		Nitrified N on total (average of percentages)		Total nitrogen (Mgms. N per 100 grms. of soil)			Average of per cent losses after 61 days	
					At start	After 61 days			
	Sun	Shade	Sun	Shade		Sun	Shade	Sun	Shade
Compost (A) . . .	3·57	3·10	4·37	3·72	81·74	74·55	72·09	8·62	11·81
Amm. Sulph. (B) . .	3·43	2·72	4·31	3·39	79·53	74·03	75·50	7·07	8·38
Control (C) . . .	2·94	3·02	4·18	4·27	70·68	60·61	59·39	11·38	14·24

(b) Comparisons (values of t)

Treatment	Sun *vs.* shade			Sun				Shade			1st *vs.* 2nd period		
	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Comparisons	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Treatment	Sun	Shade
A	5·52	4·64	3·09	A *vs.* B	2·33	0·86	1·08	1·95	1·83	1·99	A	0·12	3·35
B	3·94	4·61	0·69	A *vs.* C	7·00	1·73	2·51	0·73	3·14	1·53	B	0·98	1·03
C	0·74	0·60	1·79	B *vs.* C	5·16	1·08	2·95	1·62	5·78	0·82	C	0·19	2·05

Note.—n=14 throughout $\therefore$ t=2·145.

TABLE VIII

Percentage changes in and net values of nitrate accumulations and intensities of nitrification in each period in unwatered soils

Treatment	Accumulations (Mgms. N per 100 grms. of soil)						Intensities (Percentages of nitrified N on total)					
	Sun			Shade			Sun			Shade		
	During		Per cent decrease during 2nd period	During		Per cent decrease during 2nd period	During		Per cent decrease during 2nd period	During		Per cent decrease during 2nd period.
	1st period	2nd period		1st period	2nd period		1st period	2nd period		1st period	2nd period	
Compost . .	3·20	0·37	88·45	3·40	Loss 0·30	108·8	3·91	0·46	88·22	4·27	Loss 0·55	112·9
Amm. Sulph.	3·13	0·30	90·42	3·18	Loss 0·46	114·5	3·75	0·56	85·07	3·99	Loss 0·60	115·0
Control .	2·80	0·14	94·99	3·08	Loss 0·06	102·0	4·50	0·32	107·1	4·36	Loss 0·09	102·1

(c) *Watered and unwatered soils*

The accumulation of nitrates and the intensity of nitrification is higher in watered soils under all conditions than in dry soils (Tables IX and X *a*, *b*, *c*).

In sunlight, watered soils did not show differences due to treatments in the first period while in dry soils there was a gradation, compost standing highest followed by ammonium sulphate, control being last.

In shade in the same period ammonium sulphate topped the list in watered soils but was not significantly different from compost, while in unwatered soils there was no difference due to treatments.

During the second period both in sunlight and in shade the watered soil showed a gradation—compost standing first followed by ammonium sulphate and control. In dry soils during the same period the gradation existed only in sunlight but not in shade.

These differences in behaviour of treatments in presence and absence of water does not seem to be due simply to general differences in water contents. It will be seen from Table XI (*a* and *b*) that the moisture contents of unwatered soils were higher during the second period than in the first with the exception of control in sunlight, which did not alter. Between treatments, under all conditions, untreated soils retained more water than the treated ones. Ammonium sulphate showed the least water content, except during the second period (in both sunlight and shade) when, though numerically consistently lower, its water-content did not significantly differ from that with compost.

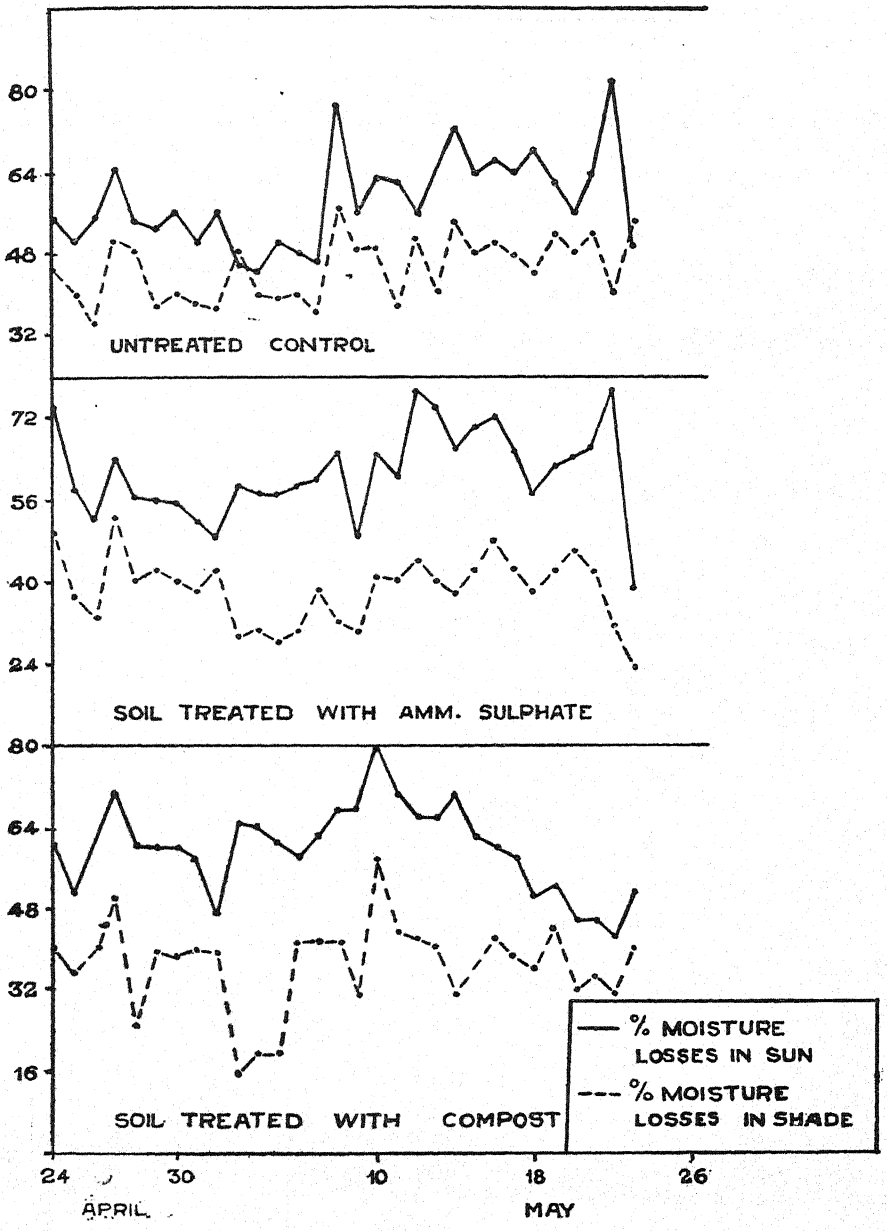

Fig. 2. Daily per cent losses of moisture from experimental soils.

The influence of time on nitrate accumulation and the intensity of nitrification in dry soils, both under sunlight and in shade is compared in Table XII (*a*, *b*, *c*).

In sunlight, in spite of the identical moisture contents of the untreated soils in both periods, these accumulated more nitrates in the second period than the first.

TABLE IX

Nitrogen changes in watered and unwatered soils during 31 days

On oven-dry basis

(*a*) *In sunlight*

Treatment	Accumulations (mgms. of N per 100 grms. of soil)		Nitrified N on total (average of percentages)		Total nitrogen				
					Mgms. N per 100 grms. of soil			Average of per cent losses after 31 days	
					At start	After 31 days			
	Watered	Unwatered	Watered	Unwatered		Watered	Unwatered	Watered	Unwatered
Compost . .	5·81	3·20	7·11	3·91	81·74	76·79	74·55	5·94	8·80
Amm. sulph. .	5·50	3·13	6·92	3·75	79·53	77·76	72·80	3·07	8·83
Control . .	5·48	2·80	7·88	4·50	70·68	58·68	62·64	16·98	11·15

(*b*) *In shade*

Compost . .	4·45	3·40	5·45	4·27	81·74	74·32	75·53	9·06	7·41
Amm. sulph .	4·55	3·18	5·72	3·99	79·53	75·54	74·94	5·20	6·41
Control . .	3·92	3·08	5·55	4·36	70·68	59·99	60·61	15·24	10·39

TABLE IX—*contd.*

(c) *Comparisons—watered* vs. *unwatered soils (values of t)*

Treatment	Sun			Shade		
	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Nitrate accumulation	Per cent nitrified N	Nitrogen losses
Compost	12·72	11·43	1·62	4·77	4·92	0·92
Amm. sulph..	15·80	15·85	3·62	6·23	6·29	0·77
Control	8·65	7·43	4·98	4·00	2·90	2·84

Note.—$n=14$ throughout, $\therefore$ $t=2\cdot145$.

TABLE X

Nitrogen changes in watered and unwatered soils during 61 days

On oven-dry basis

(*a*) *In sunlight*

Treatment	Accumulations (mgms. of N per 100 grms. of soil)		Nitrified N on total (Average of per-centages)		Total nitrogen				
					Mgms. N per 100 gms. of soil			Average of per cent losses after 61 days	
					At start	After 61 days			
	Watered	Unwatered	Watered	Unwatered		Watered	Unwatered	Watered	Unwatered
Compost . .	7·15	3·57	8·75	4·37	81·74	72·14	74·55	10·83	8·62
Amm. sulphate .	6·56	3·43	8·25	4·31	79·53	74·22	74·03	6·66	7·07
Control . .	4·56	2·94	6·45	4·18	70·68	57·38	60·61	18·81	11·38
(*b*) *In shade*									
Compost . .	5·61	3·10	6·86	3·72	81·74	75·93	72·09	7·17	11·81
Amm. sulphate .	5·24	2·72	6·59	3·39	79·53	73·48	75·50	8·06	8·38
Control . .	4·29	3·02	5·92	4·27	70·68	58·16	59·38	18·07	14·24

TABLE X—*contd.*

(c) *Comparisons—watered* vs. *unwatered soils* (*values of* t)

Treatment	Sun			Shade		
	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Nitrate accumulation	Per cent nitrified N	Nitrogen losses
Compost	27·44	26·54	1·61	19·31	16·97	4·76
Amm. sulphate	31·30	32·84	2·87	12·92	15·24	0·18
Control	10·80	11·08	7·32	10·58	5·16	3·17

Note.—$n=14$ throughout, $\therefore t=2\cdot145$.

TABLE XI

(a) *Average of percentage moistures in unwatered soils at the end of different periods*

Treatment	1st period		2nd period	
	Sun	Shade	Sun	Shade
Compost (A).	6·74	6·81	7·13	7·23
Amm. sulph. (B)	6·14	6·40	7·01	7·14
Control (C)	7·38	7·32	7·45	7·77

(b) Comparisons (values of t)

Treatment	Sun *vs.* shade		Comparisons	Sun		Shade		Treatment	1st *vs.* 2nd period	
	1st period	2nd period		1st period	2nd period	1st period	2nd period		Sun	Shade
A	0·47	1·18	A *vs.* B .	3·43	1·20	3·42	1·80	A	2·44	6·00
B	1·73	1·86	A *vs.* C .	4·00	2·91	4·25	4·91	B	7·25	6·17
C	0·46	2·46	B *vs.* C .	9·54	3·40	6·13	5·95	C	0·64	3·00

Note.—$n=14$ throughout, $\therefore t=2·145$.

TABLE XII

Nitrogen changes in unwatered soils as influenced by the duration of time (on oven-dry basis)

(a) In sunlight

Treatment	Accumulations (mgms. of N per 100 gms. of soil)		Nitrified N on total (average of percentages)		Total nitrogen				
					Mgms. N per 100 grms. of soil			Average of per cent losses at the end of	
					At start	After			
	1st period	2nd period	1st period	2nd period		1st period	2nd period	1st period	2nd period
Compost . .	3·20	3·57	3·91	4·37	81·74	74·55	74·55	8·80	8·62
Amm. sulph. .	3·13	3·43	3·75	4·31	79·53	72·80	74·03	8·83	7·07
Control . .	2·80	2·94	4·50	4·18	70·68	62·64	60·61	11·15	11·38

TABLE XII—*contd.*

(*b*) *In shade*

Compost . .	3·40	3·10	4·27	3·72	81·74	75·53	72·09	7·41	11·81
Amm. sulph .	3·18	2·72	3·99	3·39	79·53	74·94	75·50	6·41	8·38
Control . .	3·08	3·02	4·36	4·27	70·68	60·61	59·38	10·39	14·24

(*c*) *Comparisons—1st and 2nd period* (*values of* t)

Treatment	Sun			Shade		
	Nitrate accumulation	Per cent nitrified N	Nitrogen losses	Nitrate accumulation	Per cent nitrified N	Nitrogen losses
Compost	6·75	0·12	0·12	1·67	2·68	3·35
Amm. sulph.	6·00	6·79	0·98	2·00	2·22	1·03
Control	2·79	1·61	0·19	0·55	0·56	2·05

Note.—$n=14$ throughout, $\therefore t = 2·145$.

In shade both treated and untreated soils showed no differences in accumulation between the two periods though the moisture contents were higher in the second period.

When intensities of nitrification are compared, compost in sunlight and untreated soils in shade do not show th se differences to be expected from their increased water-contents in the second period. In all other cases nitrification—both accumulation and intensity—increases with the water-content of soils.

Though the accumulation in dry soils was less than in moist, its relative rate of decrease with time was much higher (Tables VI *a* and VIII *a*) except with untreated soils exposed to sunlight. None of these peculiarities in the behaviour of dry soils seems to bear any relation to the degree of nitrogen loss sustained by the soils (Tables VI *c* and VII *c*).

Temperature variations also do not seem to be connected in any way, those between the periods showing no consistency (Fig. 1 *a*, *b*). The humidities, however were consistently higher (Fig. 3) in the second period.

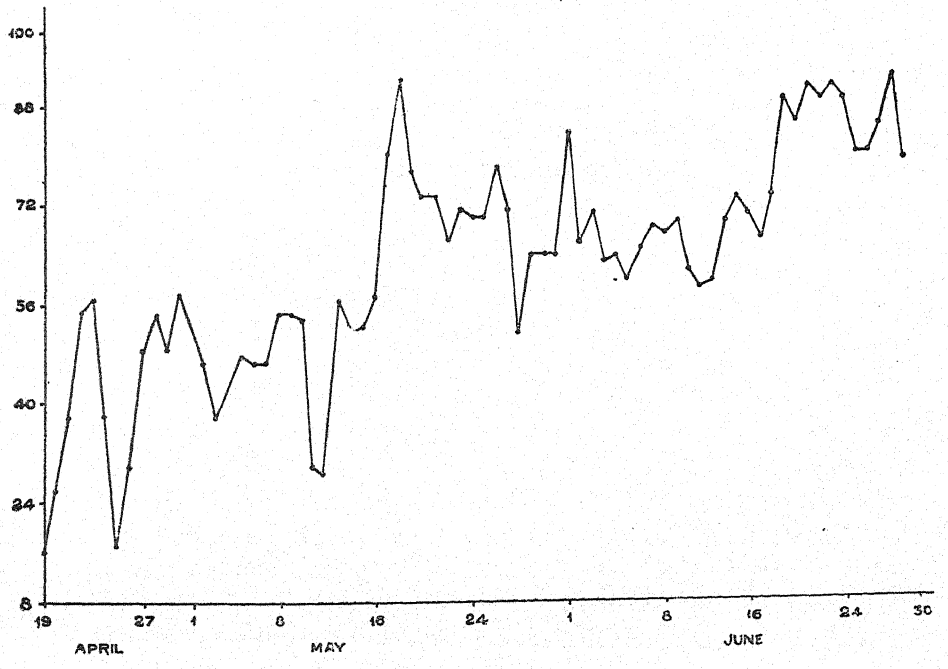

Fig. 3. Relative per cent humidities during the experimental period.

Perhaps the humidities in the air surrounding the regions of activity are of even greater importance than the actual moisture content of unwatered soils. The watered soils can obviously maintain a highly humid atmosphere even when

exposed to the sun. The unwatered treated soils, which show lower water-content than the control (and thus indicate a greater tendency to permit its evaporation) will also enable a similarly high humidity to exist around the region of nitrifying activity. The higher water-content of untreated soils seems to be less useful for this purpose, perhaps because it is less easily vaporised, being bound by the colloids to a greater extent. A possible explanation of the influence of humidity on nitrification is, that it facilitates the maintenance of a steady film of available water as a medium for nitrification, thus explaining the presence of nitrifying activity even in unwatered soils. Evidently at the surfaces of activity a moderate but sufficiently thick film of moisture must have existed. Lebedev [1930] has shown that at all moisture contents exceeding the hygroscopic amount, cent per cent humidities exist in the soil air. The maintenance of a permanent film of moisture on the colloid surfaces as small localised patches, seems thus quite possible. The limited extent of such a film is probably closely connected with the rapid decrease in nitrifying activity. It is perhaps also possible that nitrification can occur only on a certain type of clay surface which rapidly becomes unfit for the process through its own nitrifying activities. It may be that the moisture film on the colloidal clay particle permits nitrification to go on at a rate far exceeding that of the recuperative changes within that particle which retard the depression in nitrifying activity.

The comparison of exposed and covered, watered and unwatered soils lends some support to this view. With copious water supply and the stimulation associated with sunlight the deterioration was greatest but under shade it was less. With restricted water, however, the water-content which is higher in shade becomes the limiting factor, the deterioration directly varying with it (Table XI *a*, *b*). The protective influence of additional moisture in the watered soils under shade is perhaps due to the water being to a certain extent available for that process within the clay particle which retards the deterioration directly and simultaneously resulting from the nitrifying activity.

Temperature and nitrogen changes

Black cotton soil treated with ammonium sulphate equivalent to seventeen lbs. of nitrogen per acre/two inches, was kept in the shade for two months in two sets, one at an average temperature of 83·5° F. and the other at 108° F. The results are given in Table XIII.

TABLE XIII

Changes in nitrification due to temperature

	At high temperature	At low temperature	n	P	Significances
After 30 days					
Nitrate N . . .	4·55	2·20	7	$<0·01$	Highly significant.
Total N . . .	77·75	81·74	7	$>0·05$	Insignificant.
After 60 days					
Nitrate N . . .	5·24	2·30	7	$<0·01$	Highly significant.
Total N . . .	74·22	89·84	7	$>0·05$	Insignificant.

The acceleration of nitrification due to high temperatures has been recorded by Sahasrabuddhe and Kanitkar [1932]. The favourable influence of sunlight mentioned before (Tables III *a* and IV *a*), though very appreciable, falls much below that of higher temperature. Results by Fraps and Sterges [1935] show clearly that light actually inhibits nitrification, hence the greater nitrification found in the Indore experiments in trays exposed to sunlight must have been due to the higher temperature of the interior unexposed mass, the soil in the trays not having stirred to expose fresh surfaces to the inhibiting effect. As temperatures operate upon a far greater depth than light can penetrate, they must exert a far greater influence on crop-nutrition than could light. An estimate of the influence of climate, and season within the climate, on crop-development should include a relative measure of the influences of both. The practical possibility of nitrifying activity existing at high temperatures is evident from these results also.

LOSSES AND GAINS OF NITROGEN

Tables III, IV, VI and VII give losses and gains on initial total nitrogen at the end of each period.

(*a*) *Watered soils*

1st period.—Under the influence of sunlight compost treatment lost less than in shade but others showed no difference. Control lost under both light and shade much more than treated soils which showed difference only in shade, ammonium sulphate losing less than compost.

2nd period.—Compost lost more in the sun than in shade ; others showed no difference. Control lost more heavily than the treatments both in sun and shade. Only in the sun did compost show a greater loss than ammonium sulphate.

In sunlight both treatments showed a greater total loss at the end of the second period than the first. Other differences were not significant.

Table XIV *a* gives the percentages of actual losses or gains incurred during each period as well as the changes in the degree of loss expressed as the percentage increase or decrease over the loss in the first period.

TABLE XIV

Percentage losses in total nitrogen during each period in sunlight and shade with their decreases or increases between the two periods. (These losses are calculated on total nitrogen existing at the beginning of each period.)

Treatment	Sun			Shade		
	During		Per cent increase or decrease of losses on 1st period	During		Per cent increase or decrease of losses on 1st period
	1st period	2nd period		1st period	2nd period	
			(*a*) *Watered soils*			
					Gain	Decrease
Compost . .	6·06	6·06	0·00	9·08	2·10	123·40
			Increase			Decrease
Am. sulph. .	2·23	4·54	103·6	5·02	2·06	47·21
			Decrease			Decrease
Control . .	16·97	2·22	86·92	15·12	3·12	85·96
			(*b*) *Unwatered soils*			
			Decrease			Decrease
Compost . .	8·80	0·00	100·00	7·60	4·56	26·84
		Gain	Decrease		Gain	Decrease
Am. sulph. .	8·46	1·69	119·90	5·77	0·75	113·00
			Decrease			Decrease
Control . .	11·38	3·24	71·53	14·25	2·03	92.23

These figures are calculated from the means and do not represent the averages of individually-calculated results as do those in other tables where statistically examined data are included. The conclusions, however, are based on wide differences and hence are likely to be reliable.

With compost the rate of loss in sunlight is the same in both periods. Under shade there is an actual gain in the second period though the rate of loss is higher in shade than in sun during the first period. During the second period losses with ammonium sulphate increased in the sun and decreased in the shade. In the first period the order was reversed, so resembling compost. The losses in controls in the second period decreased but were practically alike in the first period and much higher than in the treatments. During the second period and in shade the controls lost more than the treatments but in the sun this was reversed. The losses with compost were mid-way between those of control and ammonium sulphate for all conditions except the second period in shade.

It is remarkable that under all conditions a period of low rate of loss is followed by one of a higher rate and *vice versa*. Compost showed a steady rate of loss under unfavourable conditions and could actually achieve a net gain in favourable conditions, while under similar environment ammonium sulphate and control showed greater fluctuation in the rates of their losses, and no gains.

This seems to lend further support to the view expressed before that two processes—one destructive and the other constructive—always go on in the soil simultaneously with the changes in its nitrogen. Organic matter, if supplied, retards degeneration and promotes recuperation. The dependence of soil efficiency on the existence of organic matter of a certain type is strongly indicated; further, in the very process of nitrogen change such organic matter is utilized.

(*b*) *Unwatered soils*

1st period.—Sunlight, shade or treatments made no difference to losses of nitrogen.

2nd period.—Compost showed a greater loss in shade, others did not show any difference. Between treatments in sunlight, control showed a greater loss in this period. Comparing the two periods, there were no differences in sunlight but in shade compost and control lost more during the second than the first period. With ammonium sulphate there was no change.

Table XIV *b* gives the percentages of actual losses or gains incurred during each period as well as the relative changes in the degree of loss expressed as the percentage increase or decrease over the loss in the first period.

Compost in the second period showed decrease in the rate of loss which decrease was intensified in sunlight. The rate of loss was slightly higher in sunlight than in shade during the first period.

Ammonium sulphate in the second period showed a similar, but more pronounced series of changes, almost alike in light and shade, so that actual gains were registered.

Control in the second period showed decreases in rate of loss both in light and shade but in the first period more so in shade, where its rate of loss was also higher than in sunlight. Control showed the highest initial rates of loss everywhere, compost coming next and ammonium sulphate being nearly equal to compost in sunlight but lower in shade.

The steadying influence of compost is again noticeable. The succession of higher rates of losses by lower ones or by gains is also apparent. Ammonium sulphate shows, however, a greater efficiency than compost in contrast with its behaviour in watered soils. This, as suggested before, seems to indicate its ability to utilise soil carbon more efficiently and perhaps also the need of sufficient moisture for the operation of compost in helping recuperation.

(c) Watered soils compared with unwatered

Tables IX and X show the comparisons.

The losses were greater with unwatered soils treated with compost in shade during the second period ; other conditions made no difference.

Ammonium sulphate in unwatered soils showed higher losses in sunlight in both periods. There were no other differences.

Untreated soils, however, showed everywhere greater losses in presence of water. Thus, under conditions of greater activity (watered soils) the treatments led to a more efficient nitrogen economy. The influence of compost is obviously through its organic matter, but that of ammonium sulphate again requires explanation. Whether it is due to more efficient use of existing carbon or to other causes it is hard to say in the absence of determinations of carbon contents at different stages.

A further point to be noted is the apparently general co-existence of high nitrification with high losses and of low nitrification with low losses. In subsequent monsoon experiments, however, (yet to be reported) the reverse was found. Even in the present experiments watered soils in sunlight, treated with ammonium sulphate lost more in the second period when accumulation of nitrates had fallen. It also happened that atmospheric humidities were high whenever losses were low and when gains were noticed. The changes in the nature of the medium (treated and untreated soils) seem to need better understanding before the differences observed can be satisfactorily explained.

Further, it appears that the ease with which soils lose water seems also to affect nitrogen losses. For instance, the daily moisture loss (from watered soils) increased, by exposure to sunlight, by 27·2, 22·5 and 12·1 per cent for compost, ammonium sulphate and no treatment respectively. The mean percentage losses in total nitrogen in sun-exposed soils decreased by 3·12 and 2·13 for compost and ammonium sulphate but increased by 1·74 for no treatment. With decreased mobility of water the tendency to lose nitrogen seems to have increased. Perhaps a difference in texture is operating.

TABLE XV

Treatment	Differences in watered soils between exposed and shaded samples during the first period	
	Daily moisture losses	Mean percentage of losses in total nitrogen
	Per cent	
Compost	+27·18	Decreased by 3·12
Amm. sulph.	+22·46	Decreased by 2·13
Control	+12·05	Increased by 1·74

DAILY PERCENTAGE LOSSES IN MOISTURE FROM WATERED SOILS

TABLE XVI

(*a*) *Average of percentage moisture losses and the differences in losses in watered soils in sunlight and shade (on fresh basis).*

Treatment	Average of daily percentage moisture losses				Differences in per cent moisture losses in sun and shade during 1st period
	During 1st period		During 2nd period		
	Sun	Shade	Sun	Shade	
Compost (A)	63·7	36·5	49·2	57·1	27·2
Amm. sulph. (B)	58·2	36·3	58·0	50·8	22·5
Control (C)	56·8	44.7	51·2	52·0	12·1

(*b*) *Comparisons (values of* t)

Treatment	Sun *vs.* shade		Comparisons	Sun		Shade		Differences in per cent moisture losses in sun and shade during 1st period
	1st period	2nd period		1st period	2nd period	1st period	2nd period	
A . . .	8·09	1·45	A *vs.* B	1·44	1·41	0·05	1·07	1·03
B . . .	6·54	0·93	A *vs.* C .	1·92	0·39	2·33	1·34	3·03
C . . .	3·27	0·23	B *vs.* C .	0·34	1·41	3·05	0·21	2·40

Note.—*n* for comparisons during 1st period=26, ∴ t=2·056.
n for comparisons during 2nd period=16, ∴ t=2·120.

During the first period the differences in daily moisture loss in exposed samples were not significant, while in shade only the untreated soils showed higher losses. This indicates better preservation of moisture by treated soils

in shade and also that when exposed they are at least not worse than control in this respect.

In treated soils whether in sun or shade moisture losses did not differ but were greater than in control; treatments hence induced a greater mobility of water. The better conservation, coupled with this greater mobility is an index of improved texture. The greater stability of water content in manured soils under natural field conditions may be explained as being the direct result of the cessation of the upward rise of the more mobile water much earlier than in unmanured soils, owing to the complete drying out of the surface due to the more open texture, further losses then ceasing. The similarity of both compost and ammonium sulphate in these respects indicates that in the very heavy intractable black soils, open texture, no matter how produced, is essential to maintain steady moisture (with its dissolved material) coupled with easy mobility of air and water, in spite of the usual weather fluctuations. The importance of this to crop-development is obvious.

During the next period the differences were not statistically significant anywhere. This is due to the high error of the treated samples in most cases. An important point however is noticeable. High humidities prevailed in this period (Fig. 3). The losses with compost-treated soils were consistently the highest (numerically) in shade but lowest when exposed. Hence the paradox that soil moisture tends to be reduced when surface evaporation is low and to be preserved when it is high. The great sensitiveness in achieving this adjustment is indicated by the high errors. The daily figures for losses fluctuated in as varied a manner as the weather changes, reducing their uniformity to a minimum. The capacity of the compost treatment to minimise the chances both of deficiencies as well as excesses of soil water is vividly brought out and its practical value needs no mention. The rôle of a loose mulch in moisture regulation seems to be capable of a similar explanation. Moisture will be retained, lost or kept constant according to atmospheric environment.

The behaviour of ammonium sulphate was just the reverse of that of compost though the sensitiveness as indicated by the magnitude of error is of the same order. The losses were low under shade and high when exposed. A tendency to accumulate water with low evaporation and to allow it to dry out when it is high is certainly not optimum for crop-development. This difference in the openness produced by minerals and that resulting from organic matter is of considerable practical and theoretical significance. The low nitrification of ammonium sulphate in spite of better nitrogen conservation in the open as compared with that of compost illustrates the point.

Moisture contents of unwatered soils at the end of each period (Table XI a, b)

In the first period when humidities were low, exposure did not make any difference to water content. Under the high humidities prevailing in the next

period, however, the untreated soils showed a higher moisture in shade, so contrasting with the treated soils. Under shade all soils contained in this period more moisture than during the first dry period. These findings indicate a slow accumulation of moisture in the lower layers of the soil during the humid period prevailing just before the advent of rain. Lebedev [1930] has observed a similar phenomenon of the condensation of atmospheric humidities inside the soil depths, and Chaptal [1934] has demonstrated it with limestone pebbles in lysimeters. The desiccation during the dry period releases more solubles [Steenkamp, 1928] but very dry black soil is not easily wetted until subsequent accumulation of condensed water in the more humid periods facilitates wetting on the advent of rains. In addition the daily moisture fluctuations in this more humid period are likely to enable the freshly released bases to enter the clay complex and improve its base-exchange equilibrium. The importance of detailed information about the period and intensities of the several stages in the seasonal recuperation of soils in any locality cannot be over-estimated. Such data alone are the means of devising rational ways for the most efficient practical utilization of the factors operating in nature.

Conclusion

Repeated failure to secure, through the action of light, nitrification in treated and untreated soils, kept sterile right up to the end, indicate the absence of any predominant photo-chemical activity. The indispensability of biological action is equally well demonstrated by the presence of nitrification whenever accidental infection occurred. The prevailing high temperatures during the experimental period also prove the possibility of micro-organic activity at least up to 46° C. The continuous existence of nitrifying activity in compost heaps themselves, after a prolonged exposure to high temperature in their initial stages, and even in the hottest surroundings of arid regions, amply supports this view.

Under natural soil conditions, however, nitrification may easily go on at much lower temperatures, being shifted from zone to zone in the soil profile. The intensity of nitrification, also, need not be always at its maximum level. The concentration of microorganic population may not always vary directly with the concentration of the products of their activity in the same zone. The vertical movement of soil moisture should be able to transport the salts from the seat of their production and accumulate them elsewhere. With an adequate understanding of the existing balance of soil processes in a locality it may, after all, be found that the principles underlying them are the same all over the world.

Biological nitrification in watered soils has the appearance of being stimulated by exposure to sunlight but other factors commonly associated with sunlight, *e.g.*, temperature, may sometimes swamp this apparent effect of light, if, indeed it exists.

Continued nitrification gradually reduces its own activity. This point is reached sooner the greater its intensity in the early stages. The steadying influence of compost on nitrification suggests the intervention of organic matter in the process. Under the stimulation by sunlight and its associated temperature rise the lower intensity with ammonium sulphate treatment, coupled with its steadying influence (similar to that of compost), appear to show that the oxidation of ammonium sulphate is brought about by the same agency which oxidises compost and not by a straight-forward chemical reaction.

The modifying influence of moisture supply and organic matter on the effect of sunlight, is well illustrated by the results obtained with dry soils.

There is an indication that the nitrifying activity depletes the soil of the essential material for optimum environment, thus, reducing nitrification with the advance of time. The existence of micro-organic activity at moisture contents very slightly in excess of the hygroscopicity of soils is to be noted. The cent per cent humidity existing in the atmosphere around the soil colloids may, perhaps, be able to maintain a limited extent of film-moisture so essential for micro-organisms. The greater activity of dry soils in the second period with prevailing high humidities in the air seems to justify this assumption. With limited moisture-contents the nitrification in the moisture films may lead to the depletion of the essential ingredients of the soil colloid feeding the organisms in the film, which then becomes unfit to maintain further nitrification.

Temperature must produce a higher degree of fluctuation in nitrification than sunlight, the former influencing the whole soil profile in contrast with the mere surface action of light.

The foregoing conclusions indicate the necessity of estimating the nitrogen nutrition of crops in terms of the resultant effect of all factors involved.

Under the conditions of the experiment, nitrogen was usually lost. There appears to be a sequence of high and low losses. Organic matter showed a steadying influence and sometimes even a gain. There was no consistency in the influence on losses or gains exerted by light and shade. The extent of decrease in subsequent losses shows a relation to the magnitude of their initial increase in soils untreated or treated with ammonium sulphate but with the compost treatment this relation does not hold good. The extent of loss is consistently less in the presence of compost and may even become a gain. The simultaneous occurrence of two independent activities seems to be indicated, one leading to losses and the other resulting in gains, the actual figures indicating a balance between the two. The nature of this balance and the co-existence or otherwise of high nitrification with high gains or high losses of nitrogen, appears to be determined by the existing intensities of the predominant factors mainly responsible for their control. The condition of the argillo-humus complex of the soil seems to be the main determinant, other environmental factors being accessories. The relative extent of aerobic and anærobic conditions in the micro-surroundings of

the complex, must obviously vary with that of its coagulated and non-coagulated components, as determined by their composition. The degree of resulting fixation, oxidation or loss of nitrogen will obviously depend upon this essential factor, further modification due to environment (such as temperature and moisture) being additional. The well-known coagulative influence of certain bases on the colloids of the soil complex should obviously play an important part in these phenomena.

Differences in the capacities to lose moisture both in sunlight and in shade bring out the superior mobility of water in treated soils. The increased mobility associated with compost enables a regulation of moisture content favourable for crop-development, moisture being easily reduced when in excess and conserved when deficient. With ammonium sulphate the reverse happens, a condition not congenial for crop-growth. This difference in open textures produced by organic and inorganic substances is noteworthy. It is suggested that a loose mulch maintained on the soil surface may operate just like compost treatment. Moisture is regulated and may be lost, conserved or remain unchanged according to the nature of the environment influencing evapcration.

Dry soils tend to accumulate moisture from the air when humidities are high. An interval of high humidities between the hot, dry season and the advent of rains seems to be an important phase in the process of soil recuperation. The heated but still dry soils with their released nutrients do not soak easily. A slow absorption of water daily fluctuating in degree owing to the weather changes brings about the even incorporation of these released nutrient materials and at the same time enables the soil on the later advent of rains to absorb larger quantities of water quickly. The value cannot be over-estimated of securing accurate information about the extent of these stages in natural soil recuperation in any locality. Means to achieve the maximum development in all these stages should form one of the main items in efficient soil management.

Summary

The nitrogen changes occurring in wet and dry soils, manured and unmanured, were estimated at high temperatures with low and high humidities, in sunlight and in shade. The relative predominance of factors involved was estimated. The results were statistically evaluated.

Results with sterile soils indicated the predominance of biological action but gave no significant evidence of photo-oxidation. The possibility of the accumulation of soil-nitrates by normal micro-organic activity under very drastic conditions is discussed.

Rise in temperature, whether due to direct sunlight or otherwise, and in moisture, intensified nitrification. Nitrogen losses occurred during these experiments with different intensities.

The all-round favourable influence of compost both on the oxidation and conservation of nitrogen under varied surroundings is emphasized. Both compost and ammonium sulphate improved soil texture but the effect on moisture conservation was not the same. The soil processes seem to be affected less by external surroundings than by the properties of the soil-complex which, however, undergoes continuous modification both by the duration of micro-organic activity and by changes in environment. It is suggested that a sound basis for improvements in soil management will be available when accurate information is secured regarding the existing balance in a locality between the several active factors and the limits to their control.

ACKNOWLEDGMENTS

With great pleasure we express our gratitude to Mr. F. K. Jackson for his assistance in drafting this paper and to Mr. J. B. Hutchinson for his suggestions regarding the tabulation of the statistically examined data in a very concise manner.

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STUDIES IN INDIAN FIBRE PLANTS

NO. 4.—THE ROOT-SYSTEM OF SUNN-HEMP (*CROTALARIA JUNCEA* L.)

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(With Plates XXVII and XXVIII)

I. INTRODUCTION

Sunn-hemp is lately being used in many places as a suitable substitute for Russian hemp (*Cannabis sativa* L.). In view of its growing importance as a fibre crop and its increased popularity as a green manure in the tropics, a detailed study of the crop is desirable. Such a study has, therefore, been taken up at Pusa with the object of breeding strains suitable for the different tracts in India.

A large number of samples of this crop was collected in 1931 from almost all the provinces in India and single plant selections were made from this material. Unlike most of the crops studied in this Section, sunn-hemp is cross-fertilised and this fact has proved a limiting factor in the isolation of pure lines, although some very promising strains have been secured by mass selection.

There is a great deal of variability in the amount of seed-setting, maturity, plant habit and in certain other morphological characters in the cultures under study. As the material was selected from seed samples collected from various parts of the country, differing widely in agricultural as well as in climatic and edaphic conditions, it was natural to expect some variability in the root-system of the cultures as well. A study of the root-system of sunn-hemp varieties was considered to be of fundamental importance and was undertaken in 1933 and 1934 at Pusa. From previous studies with other crops conducted at Pusa it

has been established that plants with a deep root-system do not suit climatic conditions usually obtained in Bihar or Bengal, and similarly plants with a shallow root system do not thrive properly in the black cotton soils of Peninsular India.

The heritable tendencies in the different types of root-system of a crop are usually found to be more powerful than the influence of environmental changes and hence a knowledge of the root habit of crops is of great value in problems of plant selections and crop adaptations. The importance of the root habit of crops has been fully realized and increased attention is now being paid by plant breeders to the study of root-systems of different crops. In India itself, Howard and Howard [1917], Venkataraman [1917], Ali Muhammad and Deshpande [1929], Sethi [1929], Kashi Ram and Madhava Rao [1931], Bose and Dixit [1931] and Bose and Joglekar [1933] as well as some others, have made contributions on the subject.

II. Material and method

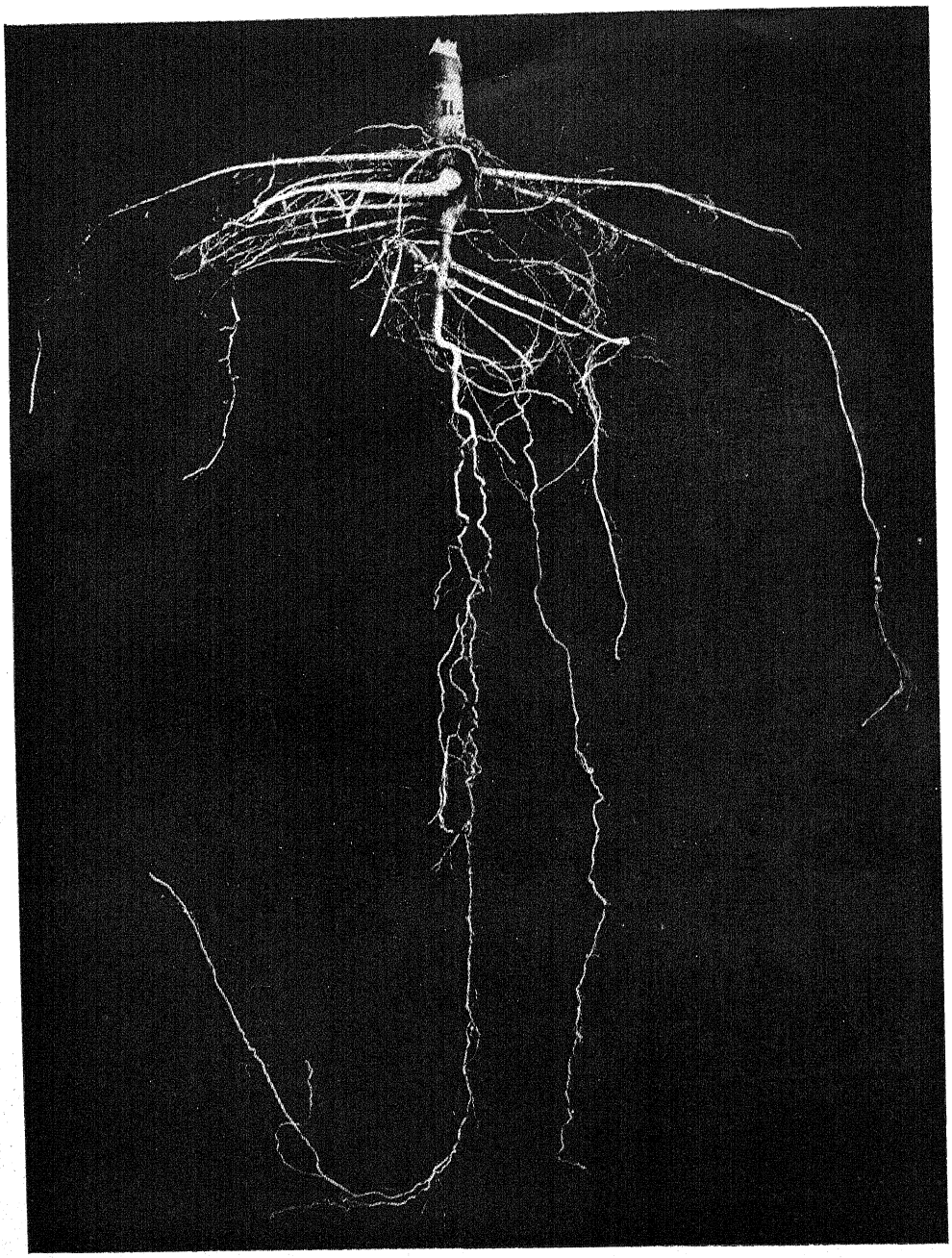
Sunn-hemp is best adapted to lighter, better-drained lands but is also capable of adapting itself to heavy soils. For the purpose of the present study the crop was grown on a well-drained and slightly clayey loam. The crop showed normal and healthy growth. A number of plants in each culture was examined each year and the averages of all measurements of the extent and distribution of roots were carefully recorded. The method of root-washing and of recording observations were the same as described in a previous publication [Bose and Dixit, 1931].

III. General description

In the year 1933 the roots of all the sunn-hemp cultures were exposed at monthly intervals, beginning one month after the date of sowing. In the case of early observations, however, no clear-cut differences could be found in the development of the main roots till quite a late period in the plant's growth. This periodic root washing showed that the largest number of root tubercles in this crop was found immediately before the flowering stage, and thus to ensure maximum benefit the crop should be ploughed in for green manuring purposes at this stage. Later than this the plants may also become too tough and may not rot so rapidly. It was interesting to note that the cultures with well-developed fibrous roots possessed the largest number of root nodules.

The clearest differentiation between the different types of root-systems is possible when the plants are fully mature, and consequently root washings were made only at this stage in 1934. The observations regarding the depth, extent of lateral spread, working depth, etc., made in 1934 supported and confirmed those made in the previous year.

PLATE XXVII.



Mesophytic type of root-system in sunn-hemp.

Sunn-hemp possesses a generalized root-system, a term which indicates that both the tap root and the laterals are very well developed. The copious branching which the roots exhibit, enables the plant to draw its nourishment from a large soil area and the roots usually penetrate the ground deeply and reach out quite widely. The tap root is strongly developed and produces the greater portion of its feeding roots in the upper few inches of the soil. The depth to which the primary roots grow is mainly determined by the drainage conditions and the nature of the soil. In sandy soils, for instance, the tap root invariably grows deeper than in heavy clay loams where it cannot penetrate so far, but under the latter conditions, the secondary roots are generally profuse and more developed. The secondary roots branch off laterally from the primary tap root and in turn produce a large number of tertiary roots. This process of branching continues until the soil is traversed by a complicated network of copiously branched roots making an excellent absorbing system. The lateral roots usually develop below the surface of the soil at a depth varying from half to three inches, but under conditions of excessive soil moisture it is not rare to find them coming up almost to the surface of the soil and these are then liable to considerable damage during inter-cultivation. After growing in a lateral direction for some distance most of the secondary roots usually bend directly downwards, the largest frequently ending in the third or fourth foot of the soil, thus ensuring an adequate supply of soil moisture. It is interesting to note that cultures with the deepest tap roots possess few laterals and form only a small number of fibrous roots and also that very few root nodules are left when the root-systems of fully matured sunn-hemp plants are exposed for examination.

The root-system of the Indian varieties of sunn-hemp may be broadly divided into two main groups, *viz.*, (1) Mesophytic and (2) Xerophytic, each of which may further be sub-divided into two sub-groups. This is in close agreement with the root-systems observed in Indian barleys [Bose and Dixit, 1931] and in green and black grams [Bose and Joglekar, 1933]. A brief description of the types of root-systems usually observed in the case of sunn-hemp is given below :—

I. Mesophytic—A. The tap roots are not very deep, growing generally upto thirty-five to forty inches in depth. There are numerous well-developed secondary roots arising almost at right angles to the tap root. The extent to which these laterals grow horizontally is fairly wide, *i.e.*, from twenty-one to forty inches. Thus they provide the plants with very strong anchorage. Fibrous roots are also very well developed (Plate XXVII).

Mesophytic—B. The tap roots reach a depth of about forty-one to forty-five inches. The secondary roots are found developing up to a greater depth and arise usually obliquely at an angle and finally take a down-ward course, the horizontal spread extending from

twenty-eight to thirty-five inches approximately. The fibrous roots are well developed.

II. Xerophytic—A. The tap root penetrates very deep, generally from forty-six to fifty inches into the soil, and frequently gives out delicate branches at a depth of about twenty to twenty-five inches. A few well-developed but sparsely branched secondary roots arise within a depth of six or seven inches of the soil and the horizontal spread is not very wide and covers a distance of about fifteen to twenty-eight inches only. The fibrous roots are poorly developed and are mostly at the lower levels of the soil (Plate XXVIII).

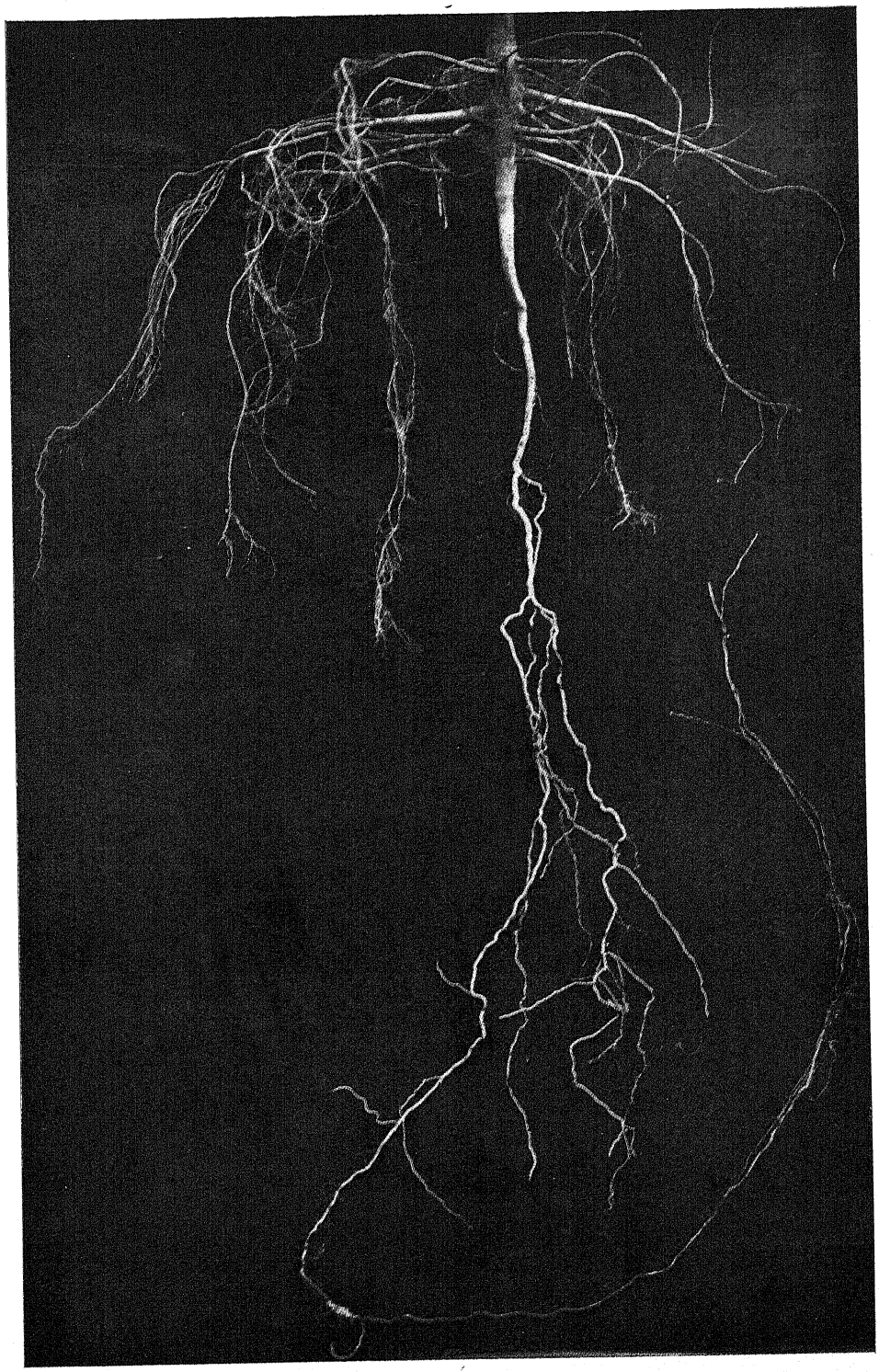
Xerophytic—B. The tap root goes down very deeply, usually more than fifty inches in depth. The secondary roots which are rather sparsely developed, arise within a few inches from the surface of the soil at a distinct angle to the tap root and almost immediately pursue a downward course. The fibrous roots are usually poorly developed and are again mostly at the lower depths of the soil.

The relevant data concerning this investigation are presented in Table I. It must be remembered however, that the measurements given in this table, as well as in the other tables, have been based on the mean values of the accumulated data for the two years under consideration. They are, therefore, not absolute but only comparative and may be expected to vary under different soil and climatic conditions. The depth of the tap root and the lateral spread of secondary roots in culture 8-2 (Xerophytic—A) in two fields in 1934 will furnish a good example of the nature of this change :—

Culture No.	Field	Nature of soil	Maximum depth of tap root in inches	Lateral spread of secondary roots in inches	No. of secondary roots
8-2 . . .	Barah South border.	Clayey loam.	47	24	6
8-2 . . .	Farm Plot 1 .	Sandy loam .	53	18	9

It is obvious that the depth of tap roots and the number of secondary roots are greater in sandy loam than in clayey soil, but the lateral spread of the secondary roots is definitely less in the former case.

PLATE XXVIII.



Xerophytic type of root-system in sunn-hemp.

TABLE I

Root-systems and some other characters in sunn-hemp cultures

Nature of root-system	Culture No.	Locality of origin		Maximum depth of tap root	Lateral spread of secondary roots	*Working depth	Development of fibrous roots	Average height of plants	Plant habit
		Province	Place	(in.)	(in.)	(in.)		(in.)	
Mesophytic—A	5-1	Assam	Gauhati	38	24	4	Fair	98	Erect
	5-2	Do.	Jorhat	39	30	4	Fair	89	Erect
	1-1	Bengal	Faridpur	40	28	5	Fair	110	Erect
	1-2	Do.	Barisal	40	24	5	Good	104	Erect
	2-3	Do.	Feni	36	28	5	Good	100	Erect
	27-2	Bihar	Pusa	40	27	5	Very good	98	Spreading
	27-1	Do.	Darbhanga	36	30	6	Very good	98	Spreading
	32-1	Do.	Muzaffarpur	40	25	6	Very good	99	Spreading
	32-2	Do.	Sepaya	36	35	6	Good	114	Spreading
	11-1	Madras	Giddalore	38	32	6	Good	106	Erect
Mesophytic—B	10-2	Madras	Giddalore	45	24	4	Good	98	Erect
Xerophytic—A	11-2	Sind	Sakrand	46	26	6	Fair	105	Erect
	11-(*a*)	Bombay	Jalgaon	48	18	6	Poor	99	Erect
	3-2	Central Provinces.	Nagpur	50	26	5	Poor	98	Erect
	30-2	Do.	Balaghat	48	22	5	Fair	118	Erect
	8-2	Central India	Indore	48	24	4	Fair	107	Erect
	16-2	United Provinces.	Aligarh	46	25	4	Fair	102	Erect
	20-2	Do.	Sandilla	48	22	4	Poor	110	Erect
	6-2	Do.	Cawnpore	51	25	4	Poor	106	Erect
	17-1	Madras	Palghat	49	21	4	Poor	110	Erect
Xerophytic—B	8-12	Central India	Mhow	52	25	6	Poor	114	Erect
	7-2	Do.	Indore	68	14	6	Fair	114	Erect
	1-(*b*)	Punjab	Multan	64	19	6	Fair	126	Erect
	11-(*b*)	Bombay	Jalgaon	56	20	6	Poor	100	Erect

* Working depth may be defined as the depth from the soil surface at which the maximum number of lateral roots are given off. In the case of sunn-hemp the variability in the working depth of the root-system is not as great as has been found in *mung* and *urid* [Bose and Joglekar, 1933].

The maximum depth of the tap root is considered as one of the chief criteria for the differentiation of the types of root system. The distribution of the different cultures according to this character is shown in Table II given below :—

TABLE II

Relation of the type of root-system to the maximum depth of the tap root

Range of maximum depth of tap roots in inches	Cultures with root-system			
	Mesophytic—A	Mesophytic—B	Xerophytic—A	Xerophytic—B
34 to 36 . . .	2-3 ; 27-1 ; 32-2.			
37 to 39 . . .	5-1 ; 5-2 ; 11-1			
40 to 42 . . .	1-1 ; 1-2 ; 27-2 ; 32-1.			
43 to 45 . . .		10-2		
46 to 48 . . .			8-2, 11-2, 11 (*a*), 16-2, 20-2, 30-2.	
49 to 51 . . .			3-2, 6-2, 17-1	
52 to 54 . . .				8-12
55 to 56 . . .				11-(*b*)
57 to 59 . . .				
60 to 62 . . .				
63 to 65 . . .				1-(*b*)
66 to 68 . . .				7-2

It is evident from the above that there is a definite rang in the maximum depths of tap roots for the different types of root-systems.

The relation of the type of root-system to the lateral spread of the secondary roots is similarly brought out in the following table :—

TABLE III

Relation of the type of root-system to the lateral spread of secondary roots

Lateral spread of secondary roots in inches	Cultures with root-system			
	Mesophytic—A	Mesophytic—B	Xerophytic—A	Xerophytic—B
12·6 to 15·0 . .				7-2
15·1 to 17·5 . .				
17·6 to 20·0 . .			11-(*a*) . .	1-(*b*) ; 11-(*b*)
20·1 to 22·5 . .			17-1 ; 20-2 ; 30-2.	
22·6 to 25·0 . .	1-2 ; 5-1 ; 32-1.	10-2	6-2 ; 8-2 ; 16-2.	8-12
25·1 to 27·5 . .	27-2 . .		3-2 ; 11-2	
27·6 to 30·0 . .	1-1 ; 2-3 ; 5-2 ; 27-1.			
30·1 to 32·5 . .	11-1 . .			
32·6 to 35·0 . .	32-2 . .			

In this case it is seen that the lateral spread of secondary roots in the mesophytic type of root-system is greater than that in the xerophytic type. Only four cultures with a spreading plant habit, *viz.*, cultures 27-1, 27-2, 32-1 and 32-2 were examined and they invariably had a larger amount of foliage development coupled with greater spread of lateral roots and also a better development of fibrous roots than the other cultures, all of which possessed erect plant habit. This of course is in conformity with the physiological fact that the plants with greater amount of foliage development require more efficient and vigorous root-systems capable of extending over a wide range of soil surface. The cultures with spreading plant habit, therefore, possess greater potentialities as green manuring crops for higher nitrogen fixation and humus formation, because of their well-developed root systems and nodule development as well as due to their heavy foliage growth. It is obvious also that the proper spacing for this crop should vary directly with the type of root-system and the extent of the lateral spread of the secondary roots.

The development of the fibrous roots and consequently of root nodules, is usually good in the cultures with mesophytic roots and mostly poor or only fair in the case of cultures with the xerophytic type of root-system. This character is of great economic importance because the greater the number of fibrous roots in a plant, the larger is the formation of root nodules containing nitrogen-fixing bacteria. Sunn-hemp strains possessing this character, therefore, should be very useful for green manuring purposes.

IV. Relation of the type of root-system to locality

The distribution of the different types of root-systems found in any crop is obviously centred round two important points, *viz.*, (1) soil æration and (2) the capacity of the soil to retain moisture. The presence of sufficient moisture and available food materials in the upper layers of the soil, as well as the anaerobic condition of the lower levels in Assam, Bengal and Bihar, or as a matter of fact in the greater part of the Gangetic alluvium, makes this tract an ideal substratum for surface rooting types. It is no wonder that the cultures with the mesophytic type of root-system, in the present studies, have originated from seed collected from these localities. Cultures 10-2 and 11-1 are exceptions, as the original seed of these strains was collected from Giddalore in the Madras Presidency. On the other hand, the drier soils of Western parts of the United Provinces of Agra and Oudh, and the greater portion of the Punjab, Sind and of Peninsular India, including Central India, the Central Provinces and the Presidencies of Madras and Bombay, with their deeper-seated supplies of moisture and better æration at the deeper levels of the soil, are adequately fitted for the growth and development of sunn-hemps possessing the xerophytic type of root-system. It is indeed from these tracts that cultures with xerophytic roots have been drawn. The

results presented in these studies, therefore, again confirm those obtained in other crops like barley, *mung* and *urid* reported before from this Section [*loc. cit.*]

Though root habit is rather responsive within certain limits to environmental conditions, its growth is primarily governed by heritable factors. In order to derive maximum benefits from improved crops, it is of fundamental importance for the plant breeder to appreciate the need for selecting plants with the proper type of root-system, suitable for the locality for which he is evolving his type. It will not do, for instance, to select for the black cotton soils of Central India a sunn-hemp variety possessing mesophytic type of roots.

V. Relation of the type of root-system to plant stature

The root-system of a plant is often said to be the image of the shoot system and frequently it is found that the taller the plant the greater is the depth of its roots. The maintenance of the proper balance between the root and the shoot of a plant is indeed of very great imporatnce ; if either is too limited or too great in extent, the other will not thrive normally. In a healthy and well developed plant the root-system must be sufficiently wide-spread to absorb enough water and nutrients for the stem and leaves, which in their turn must manufacture sufficient food for the maintenance of the root-system and for the plant as a whole.

Moderately high positive correlation ($r=0\cdot4579\pm0\cdot1088$) is found in the present studies between the average height of sunn-hemp plants and the maximum depth of their tap roots. This must not, however, be taken to indicate that all cultures which are tall must necessarily possess the deepest going tap roots or *vice versa*, for there are a number of sunn-hemp cultures at Pusa which are tall but which definitely have a mesophytic type of root-system. These cultures have a large number of secondary roots and abundant fibrous roots. In other words, the association between the type of the root-system and stature in sunn-hemp may be said to be close and the amount of root development, including the depth of the main tap root, the lateral spread of secondary roots and the nature of the tertiary roots, may be said to be proportional to the development of the above-ground parts and consequently to the stature of the plant within certain limits.

VI. Summary and conclusions

The study of the root-system of an economic crop is as important as that of its above-ground parts and the best results can be achieved from improved strains of crops only if their root-systems are efficiently adapted to the soil and climatic conditions under which they have to be grown.

At Pusa a number of sunn-hemp cultures have been evolved from material collected from different parts of India. The root-systems of twenty four cultures, representative of different tracts, have been studied for two years consecutively.

Two definite types of root-systems, *viz.*, mesophytic and xerophytic, have been recognised and differentiation between these two types has been made mostly according to the depth of the tap roots, the lateral spread of the secondary roots, and the nature and development of the fibrous roots. Further sub-divisions between mesophytic A and B and xerophytic A and B have also been made on the basis of the average depth of the tap roots.

In the mesophytic type of root-system the tap roots had lesser depths and the secondary roots had a greater spread than in the xerophytic type. Cultures which were collected from the alluviums of Assam, Bengal and Bihar and a very few others, invariably had a mesophytic type of root system, whereas cultures which were selected from seed collected from the drier localities of the United Provinces of Agra and Oudh, parts of the Punjab, Sind and Peninsular India, exhibited the xerophytic type of root-system. Moderately high positive correlation has been observed between the type of root-system and the stature of plants.

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EARLINESS IN THE UNITED PROVINCES RICES

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CONTENTS

		PAGE
I.	INTRODUCTION	361
II.	NECESSITY OF EARLY RIPENING TYPES IN U. P.	362
III.	EARLINESS IN U. P. RICES	362
IV.	CRITERION OF EARLINESS	363
V.	FLOWERING IN RELATION TO SEASONAL FACTORS AND TO THE AGE OF SEEDLINGS AT THE TIME OF TRANSPLANTING	364
VI.	FLOWERING IN RELATION TO RIPENING	370
VII.	SUMMARY AND CONCLUSIONS	375
	REFERENCES	376

I. INTRODUCTION

This note deals with some aspects of earliness in the rice crop studied during the last nine years, first at the Botanical Section, Cawnpore, and later at the Rice Research Station, Nagina. The facts described below have been collected from observations on pure lines, and are described here as a preliminary to the proper understanding of the data accumulated since 1929 on the inheritance of earliness to be described in a subsequent paper. The studies with the pure lines and the hybrid materials are the outcome of the fact that earliness is one of the most important characteristics which decide the popularity or otherwise of a variety in a locality ; hence in our programme of rice improvement in the United Provinces, adequate attention has been paid to this aspect of the crop.

II. Necessity of early-ripening types in the United Provinces

The demand for early-ripening types is great in several districts of the United Provinces. Besides enabling the cultivator to clear his field in time for the sowing of gram or wheat which generally follows paddy in rotation, early varieties make less demand on water on account of their shorter growing period. Since many parts of the United Provinces, where paddy forms an important crop, are not so well supplied with rain as other rice growing provinces like Bengal, Madras or Burma, and since the monsoon stops in September or the beginning of October, it is very important that the paddy varieties should be such as to ripen just after the withdrawal of the monsoon. Even in areas where facilities of irrigation by canal are available (and these have increased considerably in recent years due to the opening of the Sarda Canal) the earlier ripening varieties are generally preferred by the cultivators.

III. Earliness in United Provinces rices

The transplanted rices of the United Provinces can be broadly classified into early and late types. The early types flower from the end of August to the third week of September if sown in the first week of June and transplanted about four or five weeks after. They are ready for harvest in October. The late types flower from about the second week of October to the last week of the same month mostly between the 10th to 20th October, and are ready for harvest in the later half of November or in early December. This division of the United Provinces rices into two main groups is definite, very few types flowering in the intermediate period, *viz.*, the end of September or the beginning of October. The flowering of pure lines grown every year at the Rice Research Station, Nagina, which represent the majority of the rices of the United Provinces, gives a correct picture of the facts. Their flowering is shown in Figure 1. The earliest types start flowering in the end of August, *i.e.*, about eighty to ninety days after sowing, the latest in the early group taking as many as 112—115 days to flower.

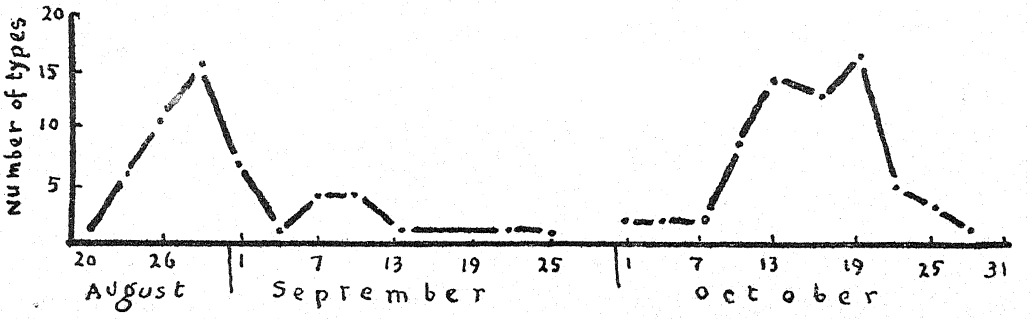

Fig. 1. Showing distribution of U. P. rices into two main groups, one early and the other late.

Among the late types the earliest to flower takes 120 days from sowing, the latest taking as many as 145 days. These figures vary slightly from year to year. The difference of the means of the two groups can be conveniently taken to be over a month. In Fig. 1 the modes are separated by about seven weeks.

Besides the transplanted rices there are a few broadcast rices. Of these, the most popular are those commonly called *Sathi dhan* (paddy). *Sathis*, as the name implies, take about sixty days to ripen after sowing. They are the earliest rices of the United Provinces, the other broadcast rices being a few to several weeks later than the *Sathis*.

IV. Criterion of earliness

Before studies upon earliness are made it has to be decided how best to judge earliness ; in other words, what should be the criterion of earliness ? The available literature on cereal crops in general shows that many workers have recognised the date of earing as the most suitable criterion for judging earliness, although other criteria have also been frequently used. Thus Farrer [1898] selected as his index the change of colour of the peduncle immediately below the spike. Biffen [1905], working on wheat, judged the fully ripe condition by the yellowing of straw and glumes and the hardening of the grain. Thompson [1918] used the term " fully ripe " when the central kernels of the wheat spike reached the dough stage. Caporn [1918] in his studies on oats considered a plant fully ripe when the last trace of greenness had disappeared from the tips of the palae. On the other hand Freeman [1919], Bryan and Pressley [1921], Florell [1924], and Allen [1924], all working on wheat, have used as the index of earliness the date of appearance of the first head on each plant.

Hoshino [1915] and Jones [1928], working on rice, selected as their index of earliness the appearance of the first spikelet above the sheath of the inflorescence. The latter says " This index of earliness was found to be more satisfactory than a record of the date when the plant is fully headed or the kernels are all ripe ".

Ayyangar [1921] used the date on which the first main head emerges out of the sheath and shows the white downy ring at the bottom of the panicle for flowering in rice, although he does not mention that he used the flowering duration as an index of earliness.

We have also used the date of emergence of the tip of the panicle above the sheath as the index of earliness. The following reasons are suggested for this :—

1. The exact date of flowering can be judged more easily and with fairer accuracy than probably any other factor like, say, the ripening of the complete ear or the yellowing of the peduncle.
2. The ripening can be abnormally affected by adverse climatic or soil conditions, but flowering is not influenced to the same extent.

Whatever criterion is used for judging earliness it should fulfil certain important conditions. It should, for example, be accurate, and reliable. As for accuracy probably no other character can be judged with greater precision and ease as the date of flowering. With regard to reliability the date of flowering is not perhaps so consistent. It may show variation from year to year, besides being affected by spacing, manurial application, age of seedlings at transplanting, etc., but even then it is probably more consistent than ripening.

In view of the importance of earliness and the necessity for caution in drawing conclusions from a study of flowering (liable as it is to variations due to the above-mentioned factors), it is proposed to examine the effects of some of the factors on flowering. We shall first consider the relation of flowering to seasonal factors, and also examine the effect on flowering of the age of seedlings at the time of transplanting.

V. Flowering in relation to seasonal factors and to the age of seedlings at the time of transplanting

The flowering dates were noted in 135 pure lines, isolated in the years from 1926 to 1929 [Sethi and Saxena, 1930], when the observations were discontinued except in the case of a few types which were used as parents for certain crosses. Observations are now available extending over seven years on eight of the pure lines, four early and four late, and it is proposed to examine them closely in order to study the variation in their flowering durations.*

* The following terms will be used in connection with the work here described.

Flowering duration is the total number of days between sowing and flowering.

Time of flowering is the particular day on which a plant is observed to begin flowering.

Flowering period is the number of days between the beginning and end of flowering in a culture.

Ripening duration is the total number of days between sowing and ripening.

Ripening period is the interval between flowering and ripening.

The first three terms are borrowed from the paper on " Inheritance of flowering duration in rice " by Ramiah [1933].

The flowering durations of the eight pure lines referred to above are given in Table I.

TABLE I

Flowering durations of eight pure lines in 1926-33

Year	Age of seedlings at transplanting	Early types				Late types			
		T. 1	T. 3	T. 7	T. 12	T. 5	T. 26	T.100	T.110
	Days								
1926 . .	43	101	103	97	99	136	129	133	136
1927 . .	33	90	95	89	88	135	130	132	134
1928 . .	36	92	90	90	94	132	125	126	131
1929 . .	30	81	93	77	84	132	120	122	130
1931 . .	36	89	102	89	95	135	126	128	131
1932 . .	32	85	96	85	86	13[illegible]	123	129	133
1933 . .	32	86	99	85	92	13[illegible]	128	129	135

It can be seen that marked variation occurs from year to year.

A close examination of the table reveals an interesting relationship between the flowering duration and the age of the seedlings at the time of transplanting. The flowering duration runs more or less parallel with the age of the seedlings, being lengthened with an increase in age. This parallelism will become more apparent when the flowering durations are represented as the averages of the four early and four late types as in Table II.

TABLE II

Variation in flowering duration with the age of seedlings at transplanting

Year	Age of seedlings at transplanting	Average flowering durations	
		Early types	Late types
	Days		
1926	43	100·00	135·50
1927	33	90·50	132·70
1928	36	91·50	128·50
1929	30	83·75	126·00
1931	36	93·75	130·00
1932	32	88·00	129·50
1933	32	90·50	132·50

If, as the figures in the above table suggest, there does exist a tendency for the flowering duration to lengthen with the age of seedlings, the bearing of this fact upon all studies of earliness will be as stated below. In breeding studies it is usual to accumulate data over a number of years in order to study the genetic variability of cultures for tracing relationship between different cultures in the same year or of different generations in different years. It is apparent, therefore, that the studies will not be quite accurate if the relationship between flowering duration and the age of seedlings is disregarded. In view of this fact it is proposed to treat the figures in Table II statistically in order to judge the reliability as well as the intensity of the relation suggested by the figures.

The relation has been tested by estimating the coefficient of correlation between the two sets of variables. The method used is the usual product moment formula :

$$r = \frac{S\,(xy)}{\sqrt{S(x^2).\,S\,(y^2)}}$$

where x and y are the differences of the variables from their respective means. The values of r for the early and late types are, respectively, $+0{\cdot}8153$ and $+0{\cdot}5320$, the two standard deviations being $\pm 0{\cdot}1368$ and $\pm 0{\cdot}2931$. For a small

sample of the size we are dealing with (*viz.*, seven pairs of observations) the value of r and its relation to its standard error cannot be depended upon to measure the significance of the correlation observed. This fact has been emphasized by Fisher [1932] who has described a special method which he terms 'transformed correlation'. According to this method the value of r is transformed into a value called z, and to facilitate the transformation Fisher has prepared a table [1932, Table VB]. Thus—

when $r=0\cdot8144$, $z=1\cdot14$
when $r=0\cdot8178$, $z=1\cdot15$

By interpolation we find that for $r=0\cdot8153$, $z=1\cdot1427$. The standard error of z calculated according to the formula—

$$\sigma_z = \frac{1}{\sqrt{n-3}}$$

is $0\cdot5$. The ratio of $z : \sigma_z$ in the present case is $2\cdot2854$, which is interpreted as a normal deviate. Now, from tables of normal deviates we find that the deviation in the normal distribution in terms of the standard error should be as follows :—

For $P=0\cdot01$ deviation should be $2\cdot575829$
$P=0\cdot02$, ,, ,, $2\cdot326348$
$P=0\cdot03$, ,, ,, $2\cdot170090$

Our value of $z : \sigma_z$ is $2\cdot2854$ which is between $0\cdot02$ and $0\cdot03$ points of P.

Thus the value of r for the early types can be regarded as significant. In other words, the coefficient of correlation, $+0\cdot8153$, may be regarded as sufficiently high to indicate a relationship between the age of seedlings and the date of flowering in the early types. We do not know whether it is the factor of the age of seedling or the climatic conditions obtaining at the time of transplanting which has given to the figures a correlational semblance. That there is something more than accident in this relationship is what we were led to suspect in the course of the last few years' breeding work, even before the figures were statistically analysed. The observations on late cultures also led to the conclusions confirmed above, *viz.*, that delayed transplanting does not delay flowering to the same extent as it does in the case of early cultures.

An experiment in randomized blocks was laid down in 1934 to test the above finding regarding the age of seedlings and flowering duration. The average flowering date of each of the plots was noted during the course of field observations.

The ages of the seedlings used at transplanting were fifteen, twenty-five, thirty-five and forty-five days. Transplanting of the different treatments was done on the same day. The results obtained were as follows :

Age of seedlings	Average flowering duration
15 days	79 days
25 days	87 days
35 days	98 days
45 days	109 days

Thus the flowering duration has been remarkably affected by the age of the seedlings.

The above fact also finds support from the flowering data collected from some of the pure lines in 1933. One of these types was transplanted on different dates from seed sown on the same date. The frequency distributions are given in Table III.

TABTE III

Frequency distributions of flowering duration of T. 1 transplanted on different dates in 1933

Flowering duration in days

Date of sowing	Date of trans-planting	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	Mean	Standard deviation	Coefficient of variation
3rd June .	3rd July .	1	...	7	2	19	7	5	7	3	5	...	2	...	...	...	...	...	...	...	86·02	2·36	2·74
Do. .	Do. .	1	1	3	1	24	17	17	4	7	9	...	1	...	...	...	...	...	...	...	86·52	2·01	2·32
Do. .	4th July .	...	...	...	...	1	5	5	4	12	11	25	11	4	5	2	...	...	...	...	90·34	2·16	2·39
Do. .	6th July .	...	...	...	2	...	10	9	26	7	27	18	10	3	4	2	...	...	...	...	89·45	2·21	2·47
Do. .	Do. .	...	...	1	...	...	15	11	29	13	12	7	...	7	...	...	...	...	...	...	88·49	1·96	2·21
Do. .	Do. .	...	...	...	...	...	3	1	15	21	21	13	6	3	2	1	...	...	...	...	89·78	1·70	1·89

It will be seen that the mean flowering duration varies slightly with the date of transplanting.

In view of the relation between flowering and the age of seedlings at transplanting it becomes quite clear how important it is in experiments, where a comparison has to be made of earliness in various cultures, that transplanting be confined within as narrow a range of time as possible. Usually at a research station the number of such cultures is large and the transplanting has to be carried out over a number of days, especially when heavy monsoon showers interfere with speedy work. This is likely to affect the flowering. Hence an attempt should be made to transplant cultures, whose flowering durations have to be accurately compared on the same date, and towards this end all the resources of the station should be employed.

Very much delayed transplanting was observed to have a curious effect on the flowering of some of the early types in 1934. Some of the seedlings of these early types were transplanted very late (about two or three weeks after the normal time) in order to fill in gaps caused by seedling mortality. These seedlings were obtained from the original nursery. The original transplanted seedlings pursued a normal course of development, each forming several tillers before commencing flowering. The later transplanted seedlings, however, consisting in most cases of a single tiller from the nursery started bearing thin poor ears within a few days of transplanting. This hastening of flowering was probably due to the plants having enjoyed a long uninterrupted growth in the nursery where they had already attained a high stage of development and were ready for flowering when uprooted, so that as soon as they were transplanted they formed ears. The earlier transplanted crop on the other hand, having received a check due to transplantation earlier in life proceeded with fresh vegetative activity before entering the flowering phase. Since the second transplanting was delayed abnormally the hastening of flowering cannot be said to be an effect of " age of seedlings " but is perhaps an expression of the ' periodicity ' of the crop. In these plants the real flowering took place when they renewed their vegetative activity after the first tiller had matured and the subsequent tillers flowered appreciably later than the rest of the crop from the first transplanting.

VI. Flowering in relation to ripening

In the study of flowering as an index of earliness we should not overlook the relation of flowering to ripening, because it is the latter phase which is most important to the cultivator. We shall therefore see whether the time which elapses between the flowering and ripening (*i.e.*, the ripening period) is constant for all the varieties, and also, whether in a particular variety it varies from year to year. This will show us how far earliness in flowering is a true index of earliness in ripening, and how far the former character can be relied upon in judging the latter.

It has been found that for a particular variety the ripening period is more or less constant for a particular locality. Slight variations may, however, be caused from year to year by climatic factors. Further, early varieties have, on the whole, shorter ripening periods than late ones. In other words, the former take less time to ripen after flowering is over than the latter. This fact is brought out clearly in Fig. 2, where the thick lines represent the dates of flowering and of ripening in some of the early and late pure lines. Two facts are clear from the graphs :—

1. The ripening period does not vary much within the group of early or late types, the ripening tending to vary with flowering. This is evident from the tendency of the flowering and ripening lines to remain parallel.
2. The ripening period of late types is distinctly longer than that of the early types. This is seen from the wider space separating the two lines in the former.

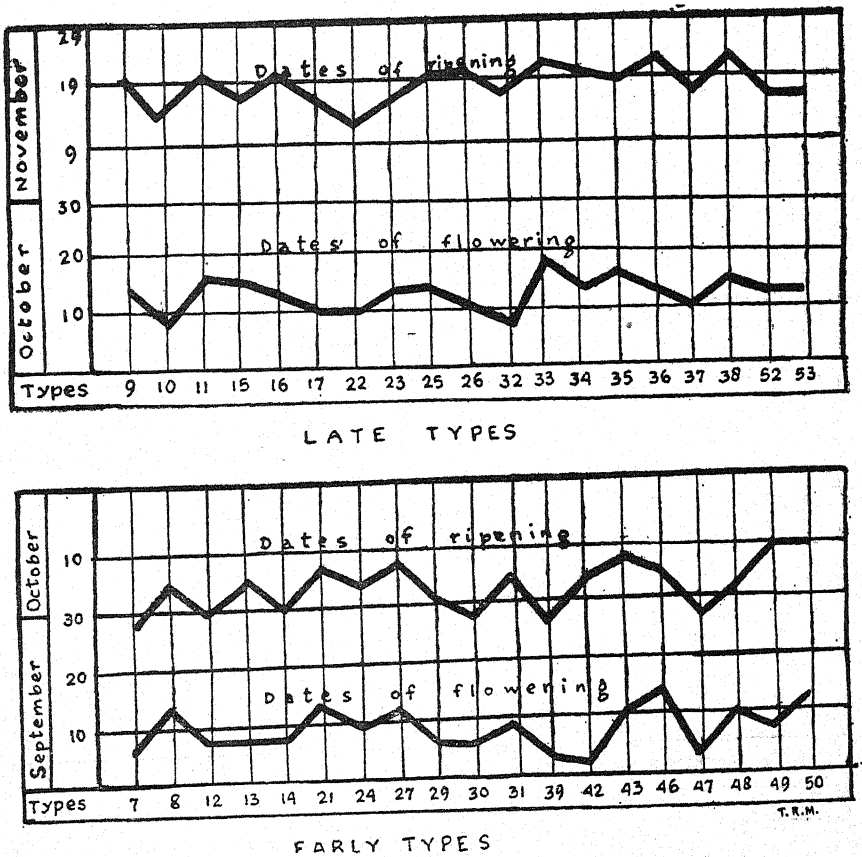

Fig. 2. Showing the ripening period in early and late type in 1928.

The latter fact was mentioned in an earlier publication [Sethi and Saxena, 1930], but the data had not been studied systematically. The data have since been closely examined and now enable us to arrive at definite conclusions. Three years' data (1926-28) on about 120 pure lines have been analysed. These pure lines comprise both early as well as late types. For each group the average flowering and ripening durations† were calculated, and the averages examined statistically. The data are summarised in Table IV.

TABLE IV

Flowering and ripening durations and ripening periods in early and late types in 1926-28

Year	Nature of types	No. of types	Mean flowering duration (A)	Mean ripening duration (B)	Mean ripening period (B—A)	Variance of ripening period	Mean difference between ripening periods of early and late types	$t=\frac{\text{mean difference}}{\text{S.E. of mean difference}}$
1	2	3	4	5	6	7	8	9
1926 .	Early	53	102·62	130·43	27·81	16·46	12·09	16·20
	Late .	63	133·02	172·92	39·90	15·51		
1927 .	Early	54	92·93	120·43	27·50	12·03	7·99	12·54
	Late .	67	132·76	168·25	35·49	12·28		
1928 .	Early	53	94·58	121·50	26·92	15·30	8·13	11·32
	Late .	66	128·24	163·29	35·05	15·02		

* The values of 't' for 0·01 point of P for populations of 30 and infinity are, respectively, 2·750 and 2·5782. The observed values of 't' are markedly higher than these.

The differences between the ripening periods of the early and the late types in 1926, 1927 and 1928 are 12·09, 7·99 and 8·13 days, respectively. These figures represent averages of over fifty types (Table IV). The differences have been statistically examined and are found to be significant. The method used is Fisher's 't'-test [Fisher, 1932] devised for comparison of mean values based on small samples. The formula is—

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}}}$$

† It has already been stated that ripening cannot be noted with the same accuracy or reliability as flowering. The ripening durations mentioned here are therefore rough approximations, arrived at after close examination of the panicles for maturity which necessitated greater care and more attention than the noting of flowering date usually does.

where $\bar{x}_1$ and $\bar{x}_2$ are the two means, S_1^2 and S_2^2 the estimates of two variances and N_1 and N_2 are the sizes of the samples.

The calculation of 't' for 1926 data is given below :—

$$t = \frac{12 \cdot 09}{\sqrt{\frac{16 \cdot 46}{53} + \frac{15 \cdot 51}{63}}}$$

$$= \frac{12 \cdot 09}{0 \cdot 7462} = 16 \cdot 20$$

This value and the values of 't' for 1927 and 1928 are higher than the values required for 0·01 probability. We may therefore state that the relation of flowering to ripening is not identical in the early and late types, because the ripening periods in the two cases are different. This fact affects the study of earliness thus. If two types, one early and one late, have a difference of, say, forty days in flowering, they will be ready for harvest with a difference, not of forty days, but longer. The latter, when compared with the former only on the basis of the flowering duration will, therefore, appear more precocious than it really is. This remark may equally well apply to early and late segregates of the crosses to be described in a subsequent paper. Thus flowering, possessing as it does different indicative values for early as compared to the late types, loses some of its merit of being the best criterion of earliness.

The examination of the statistics of the flowering and ripening of the pure lines has further enabled us to test whether within the same group of types (*i.e.*, early or late group) the types which flower earlier have shorter ripening periods. It is evident that such a relation will best be revealed by computing the coefficient of correlation between the flowering durations and the ripening periods. This was done separately for the years 1926, 1927 and 1928 for early and late types considered separately as well as together. The nine values of the coefficients of correlation are given in Table V, with their standard errors.

TABLE V

Values of coefficient of correlation (r) *between flowering duration and ripening period in early and late types*

Year	Description of group	No. of types	r	σr
1926	Early types	53	+0·283	0·1276
	Late types	63	+0·261	0·1184
	Early and late types	116	+0·496	0·0700
1927	Early types	54	—0·057	0·1369
	Late types	67	+0·103	0·1218
	Early and late types	121	+0·795	0·0335
1928	Early types	53	+0·160	0·1351
	Late types	66	+0·228	0·1176
	Early and late types	119	+0·730	0·0428

The values of r (Table V) show that the correlations are low and, in most cases, insignificant where the early or the late group is considered separately. That is, within a group, the ripening period is not necessarily greater in a type which has a larger flowering duration than in one with a shorter duration. On the other hand, when both the groups are considered together the correlations are always positive, close and significant. The facts can be interpreted thus. The low correlation within each group indicates that within a group the types may be considered genetically alike so far as the relation between their flowering durations and ripening periods is concerned. The high correlations when the two groups are considered together indicate that these groups are genetically different, the shorter duration group (*i.e.*, the early types) possessing a shorter ripening period than the other group.

It was surmised in a previous publication [Sethi and Saxena, 1930] that the difference in the ripening periods of early and late types may be due to the gradual fall in temperature from August onwards. The temperature by the end of September or the beginning of October, when the early types mature, is about 98° F.,

while about the beginning or middle of November, the time of maturity of late types, it ranges between 80° and 84° F. While there is a possibility of such an influence operating, it is felt that without controlled experiments in green houses this view is open to criticism. It may be that the fall in temperature is not the prime cause, but that the growth factors which determine flowering duration also determine the ripening period. Thus we may reasonably expect that if one phase of the life of the late types is longer (*viz.*, the flowering duration) the next phase (*viz.*, the ripening period) will also be longer than the corresponding phases of the early types. This would be but an instance of what Webber [1906] and Collins [1916] call physiological correlation, *i.e.*, the two characters in question ' are the result of the same physiological tendency. '

That long-flowering-duration varieties take a comparatively longer time for ripening than the early ones, has also been mentioned by K. Ramiah in *Madras Agricultural Journal*, Volume XV, 1927, pages 47-48.

VII. Summary and conclusions

The above paragraphs discuss some of the prominent features of the rice varieties of United Provinces as regards their earliness. They are—

1. The transplanted rice varieties can be classified into two main groups, *i.e.*, early and late.

2. The broadcast varieties contain some of the earliest types, the most popular of which are the *sathi* rices.

3. In the early transplanted varieties the flowering duration has been found to be affected by the age of seedlings at the time of transplanting, increased age resulting in delayed flowering. This has been demonstrated statistically.

4. In the case of the late varieties the age of seedlings at the time of transplanting does not affect the flowering significantly.

5. In view of the fact mentioned in paragraph 3 it is suggested that where a comparison has to be made of earliness in various cultures attempt should be made to transplant seedlings of the same age by confining the transplanting operations within the shortest possible range of time if the cultures have been sown on the same date in the nursery.

6. Very much delayed transplanting in some early types has been found to result in a precocious flowering which, however, does not represent the normal flowering phase of the varieties.

7. The ripening period is shorter in the case of early varieties than in the late ones.

8. Within the same group of varieties (*i.e.*, early or late) the relation between flowering duration and ripening period is not constant and shows insignificant correlation.

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A PRELIMINARY NOTE ON COTTON FAILURE IN THE PUNJAB AND SOME ABNORMALITIES IN THE PLANT

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(With Plates XXIX—XXXI)

Introduction

The American cotton crop in the Punjab is subject to periodic partial failures. Such partial failures occurred in the year 1919, 1920, 1921, 1926, 1927 and 1928. The symptoms of the failures were early reddening of the leaves, bad opening of the bolls with immature seeds and low quality of lint and in the extreme cases the dwarfing of the plant. Naturally attempts were made to determine the causes of such failures of the crop in these years when the yield of seed cotton was abnormally low. Various views were advanced like the heat-storke theory of Milne [1924], unfavourable combination of climatic and soil factors by Trought [1931], attack of White-fly [Roberts, 1929], Jassid attacks and concentration of salts surrounding the feeding zones of the roots. All these theories could not be supported with sufficient scientific data. It was, therefore, thought that internal nutritional disorders were perhaps the causes of such failures resulting in abnormally low yields. So the present scheme submitted by the Punjab Agricultural Department was sanctioned by the Indian Central Cotton Committee to study the physiology of the cotton plant with a view to determine the nature of the nutritional disorder produced and the causes underlying it. It was also proposed to study the growth of the cotton plant under different conditions of temperature humidity and soil factors.

On reviewing the work done on the problem and studying the evidence collected two lines of investigations suggested themselves. During the failure years the first symptoms were the reddening and bronzing of the leaves at the reproductive stage. These symptoms were a constant feature of the crop during the failure years always at the reproductive stage. It was, therefore, considered desirable to determine if the nutritional disorder was caused by the deficiency of minerals like magnesium, manganese or iron, since it has been shown in the case of other crops like corn [Jones, 1929], tobacco [McMurtrey Bacon and Moss, 1923;

1930], [Jacobson and Swanback, 1932], [Conner, 1932], oats [Piper, 1931], soy beans [Mann, 1930], [Odland and Crandall, 1932], [Odland and Knoblauch, 1934; 1935] and many others that deficiency of any of these minerals or their unavailability due to the alkaline conditions of the soils may bring about the chlorotic condition of the leaves ultimately producing the reddening effects in some cases and reducing the yields. The difficult task was to determine the dose of such minerals per acre as such experiments had not been done before on the College Farm. However, experiments were arranged on the randomised plot basis in five replications at two places, one at the Lyallpur Farm and the other at the Risalewala Seed Farm. The results of these experiments will be communicated later, but as far as it could be judged from the condition of the crop, no significant differences in the yields under various treatments are expected.

Side by side, the complete mineral analysis of 4 F and the *desi* (Mollisoni) cotton plants at fortnightly intervals was also undertaken. The pH values of the soil at different depths before and after manuring with the minerals in the field trials described above are also being determined.

The second line of investigation that suggested itself was a study of the internal structure of the plant and a thorough microchemical examination of the tissues of the roots, stems and leaves throughout the season. By such microchemical study it might be possible to notice the changes occurring in the plant leading ultimately to malnutrition of the plant.

The microchemical investigation of the cotton plant has led to important discoveries. It will be reckoned in future an important event in the history of the cotton crop in India when the microscope was first focussed on the section of a leaf discerning the abnormal features to be described below. One by one these abnormal features came to light as the season advanced and it was found that the cotton plant is afflicted by an internal trouble not known before.

This trouble is not confined to American cottons (*G. hirsutum*) alone but is equally widespread in the indigenous types. It is the time of its incidence and its intensity that bring about such wide variations in the yields from plant to plant, field to field and season to season.

One of the main reasons why the trouble has remained unnoticed is the external green and healthy appearance of the plant, though internally ' diseased '. The indigenous types do not show any unhealthy appearance but the American types fortunately show externally the effects of this internal disease in reddening of the leaves and bad opening of the bolls. When the disease takes hold of the plant at a very early stage in the growth of the plant, these external symptoms in the American cottons may be aggravated and partial or complete failure may result. The abnormal features will be first described and an attempt will then be made to connect up these abnormalities. It may be stated here that these abnormalities found in the leaves and roots of cotton plants have not been described before for any other plant.

FIG. 1. Transverse section of leaf of 4-F American, showing normal chloroplasts.

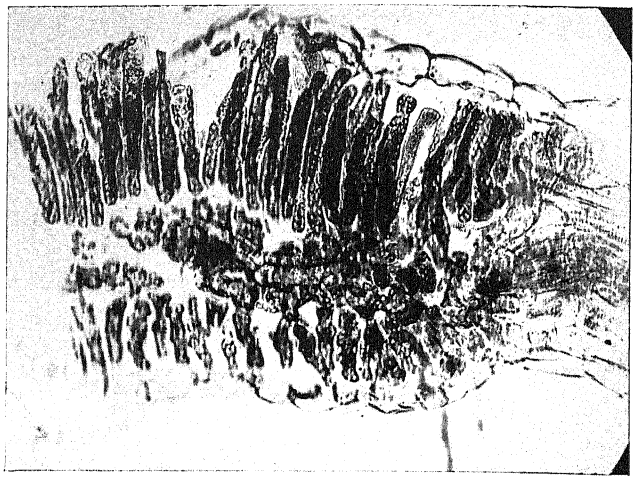

FIG. 2. Transverse section of leaf showing the disintegrated plastids.

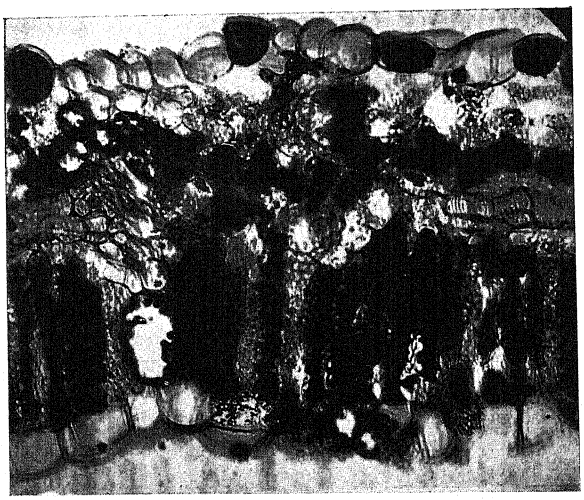

FIG. 3. Transverse section of leaf of 4-F American, showing the cells filled with yellow deposits.

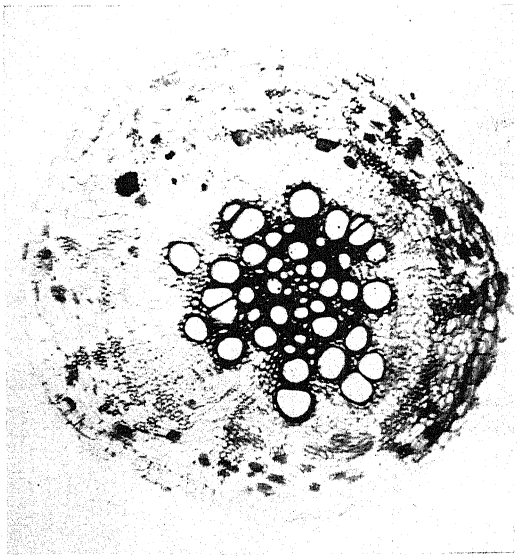

FIG. 4. Transverse section of root of 4-F American, showing the formation of colonies of particles exhibiting brownian movement.

ACKNOWLEDGMENT

I take this opportunity of expressing my deep sense of gratitude to H. R. Stewart, Esquire, Director of Agriculture, Punjab, for giving me all facilities and entire freedom to plan and to carry out this investigation in the manner I thought best. I have also to thank S. M. Nasir, Esquire, of the Punjab Agricultural College, for the bacteriological part of the investigation that he has carried out for me.

Investigation

I. ABNORMALITIES IN THE LEAVES

(1) Disintegration of the chloroplasts

In the beginning of July* while examining the tissues of the leaves of the 4-F cotton it was discovered that the green plastids in some of the palisade and spongy cells of the leaves had become broken down into small particles and in place of large egg-shaped chloroplasts arranged in parallel rows (Plate XXIX, fig. 1) the cells were filled with powdered grains (Plate XXIX, fig. 2). This observation at once struck one as being an abnormal feature; so sections of mature, healthy and green leaves were examined and it was found that in some cases all the mesophyll cells contained the plastids intact (Plate XXIX, fig. 1), in some cases some of the mesophyll cells had the chloroplasts intact, while others were broken up and in some leaves all the cells showed disintegrated chloroplasts. These three different stages in the state of plastids are hereafter termed as normal, partial and total respectively.

The leaves of different strains like the L. S. S., 43 F, 38 F, Egyptian cotton Boss III, 289 F, and 285 F were examined and similar features noted.

By the beginning of August, the number of leaves showing these disintegrated plastids increased, so much so that leaves with normal chloroplasts became rare and unusual. Towards the end of August it was not possible to find leaves with normal plastids. Even though the leaves appeared normal, healthy and green externally, yet internally they were all diseased in the sense that their photosynthetic mechanism had been thrown out of gear. In the young leaves very careful examination showed that the normal chloroplasts were absent. Whether the chloroplasts first developed and then disintegrated or did not develop at all was difficult to determine. Though the number of leaves examined every day had to be limited for want of assistance, it can be said with certainty that leaves with normal chloroplasts were rare. Plants were then obtained from fields outside the Lyallpur Farm with similar results. Plants were next examined from outside Lyallpur district but normal leaves were not found. From Shahpur district, the August specimens had leaves with normal chloroplasts but subsequent specimens obtained in September had diseased leaves. American cotton plants were obtained from Sind. In the samples sent in August, the leaves were quite normal but the samples obtained in September showed the same abnormality.

* Crop is sown in 2nd week of May and harvested in October to December.

The search was not confined to American cottons alone but to *desi* varieties also. Disintegration of chloroplasts in the month of September was noticed in the various varieties of *desi* cottons in the Punjab, Sind, Surat, Dharwar and Indore. The following table will give an idea of the different strains of the American and *desi* cottons examined and the months in which the chloroplasts were found to be broken up. In many cases the samples could not be examined earlier than the months indicated and so it is difficult to say when the first symptoms of the trouble appeared. Thus the disintegration of the chloroplasts is not a feature confined to American cottons alone but is also a common feature of the indigenous varieties so far examined.

The leaves of Verum and Buri cottons obtained from Nagpur as late as December had normal chloroplasts. Similarly the specimens received from Ujjain at the same time had partially decomposed chloroplasts in the leaves.

(2) Accumulation of starch in the leaves

The accumulation of starch is another abnormal feature noticed in the leaves. It was first noticed in the 4 F plant in August. The starch grains were large and could be easily mistaken for chloroplasts. These starch grains were first mistaken for the spheres described by Sorokin [1927] in the case of tomato maosaic. Like these spheres they were soluble in ten per cent potassium hydroxide but later they were identified as starch grains. The starch grains are large and they completely fill the palisade and spongy cells. Surrounding the starch grains are found small bodies. They form a ring round the starch grains and can be easily distinguished from the starch grains by proper staining. Under normal conditions the starch grains are always found on the surface of the chloroplasts but here they lie free in the cell and completely fill them. Under normal conditions the starch grains would be hydrolised by diastases and translocated as sugars to the interior of the plant. For some reasons or the other this does not happen in these leaves.

The accumulated starch remains in the leaf even when it has turned yellow or red. Under normal conditions the functionless leaf is depleted of starch while in the case of the American cottons examined the dead leaves in some cases contain starch. It is at present difficult to state if this is a common feature of all the dead leaves of American cottons.

The greater accumulation of starch always occurs in the leaves produced earlier in the vegetative period. In the new leaves which develop later, the starch accumulation becomes less and less because the disintegrating process in the chloroplasts sets in earlier as the season advances and consequently the photosynthetic process is also inhibited earlier in the newly developed leaves. Hence starch accumulation does not occur in the leaves developed later.

(3) Hypertrophy of the mesophyll cells

The elongation of the palisade and spongy cells is another abnormal feature of the leaves of the American cottons which manifest itself in their crumpling,

curling and thickening. The elongation of the palisade cells to nearly twice their normal length results in increasing the thickness of the leaf as a whole. The elongation of the spongy cells which is in a plane at right angles to the plane of elongation of the palisade cells increases the area of the lower surface of the leaf. Upward curling of the leaf results as the lower surface becomes greater than the upper surface. When the elongation of the palisade and the spongy cells is irregular, the leaf crumples. The elongation of the palisade cells in the leaves of the American cotton has also been noticed by Jones and Mason [1926] in two obscure diseases of cottons.

(4) Formation of tannins and other deposists in the cells

Later on the palisade and spongy cells of the leaves become filled with a yellowish mass. The plastids of the disintegrated chloroplasts disappear entirely and the starch grains usually disappear or are covered by the yellow substance (Plate XXIX, fig. 3). Some of the mesophyll cells as well as the conducting parenchymatous cells in the leaves become filled with small bodies which are round and yellow in colour. These bodies ultimately fuse together and give rise to a homogeneous yellow mass. In the later stages all the cells of the leaves including the conducting elements become completely filled with the yellow substance. The yellow mass gives the characteristic reactions of tannins, fats and protein substances. These yellow contents stain dark blue with Methyl violet, Gentian violet and many other vital stains. The whole section of the leaf looks like a row of dark cells easily seen under a low power microscope. The difference between an healthy leaf and the diseased leaf becomes most marked at this stage. Yet, then, the leaf may appear externally green in colour. A yellowed or reddened leaf has also similar deposists in the mesophyll cells.

(5) Reddening of the leaves

The reddening of the leaves is the final external symptom of the series of changes described above, and beginning weeks before the red colour appears in the epidermal cells. It is the final stage in the process of internal disintegration that occurs in the leaves. The red colour as is well known is caused by the production of anthocyanin pigments that develop in the leaves which are in the senescent stage. The acidic reaction of the cell sap gives reddish colouration to the anthocyanin pigments. There is a mixture of anthocyanins and anthoxanthins in the epidermal cells as can be seen by the change of reddish to greenish colour in the cells when they are immersed in alkaline solutions. (Anthocyanin pigments when present alone turn bluish in an alkaline medium). The production of anthocyanins in leaves of plants is closely associated with the presence of carbohydrates and this is actually the case in the leaves of cotton plant. The leaves contain starch and perhaps to some extent sugars and as they are not translocated, they give rise to anthocyanin pigments in the epidermal cells. Often anthocyanin pigments are not produced and though the green colour of the leaf is maintained,

the interior of the leaf is as much destroyed as in the case of a red leaf. A plant may have greenish leaves and yet it may be as much diseased as a plant with red leaves. Reddening is not always the external indicator of the internal trouble.

To sum up briefly, the disintegration of the chloroplasts begins to occur in the leaves. The period when it first makes its appearance appears to vary from plant to plant, field to field and perhaps from season to season and place to place. It may start at any time in July to October if the seeds are sown in May, *i.e.*, two months after sowing. Thus the synthetic mechanism of the plant becomes less and less efficient as growth proceeds till it stops altogether. There is also unusual accumulation of starch in the cells of the leaves. The accumulation diminishes in the leaves that develop at a later stage. There is also an elongation of the palisade and spongy cells in different planes and this gives rise to crumpling, curling and thickening. Later tannins develop and protein substances are produced. Finally the development of anthocyanins and anthoxanthins occurs in the epidermal cells and produces reddening of the leaves. This last change does not always take place.

A list of the various strains of American and desi *cottons examined for 'internal trouble' in the leaves, roots, and stems*

Strain	Date of collection	Remarks
4F (Lyallpur) . .	Throughout the growing season	Up to June :—Free. July : —Traces August, September } Medium Later on the trouble has been progressively increasing. *Note* (1) At Lyallpur the trouble was first noticed in a serious form in squares No. 43 and 26, Agricultural Farm, Lyallpur. (2) In square Nos. 24 and 46 the plants are healthy even in November. They are green and show good opening. Only some traces of the disease are seen inside the plant.
L. S. S. (Lyallpur) .	July and August .	Medium.
289 F. (Lyallpur) . .	11th October 1935 .	Diseased.

Free = No disintegration of chloroplasts and no deposits in the cells of the roots and stems.

Medium = Disintegration of chloroplasts occurs late in the season. The deposits appear late in the roots and stems and do not extend into the medullary rays and xylem parenchyma.

Diseased = Disintegration of chloroplasts within three months after sowing and formation of deposits in all the storage and conducting cells.

A list of the various strains of American and desi *cottons examined for interna trouble in the leaves, roots and stems*—contd.

Strain	Date of collection	Remarks
38 F	September and October	Medium
47 F	4th October 1935	Do.
73 F	11th October 1935	Diseased
43 F	7th October 1935	Do.
46 F	10th October 1935	Do.
56 F	Do.	Do.
58 F	Do.	Do.
48 F	9th October 1935	Do.
72 F	Do.	Do.
2/35 P 24	8th November 1935	Medium
1/35 P 38	Do.	Do.
10/35 P 26	Do.	Do.
22/35 P 11	Do.	Do.
8/35	Do.	Do.
9/35	Do.	Do.
43/35	14th November 1935	Diseased
44/35	Do.	Do.
29/35	Do.	Do.

Cottons from other parts of the Province

Place	Strain	Date of collection	Remarks
Kalara (Shahpur)	4 F	15th Aug. 1935	Free
		15th Sept. 1935	Diseased
		15th Oct. 1935	Do.

Cottons from other parts of the Province—contd.

Place	Strain	Date of collection	Remarks
Jaranwala . . .	4 F . . .	16th Aug. 1935 .	Diseased.
		26th Aug. 1935 .	Do.
Jahanabad (Shahpur) .	4 F . . .	14th Aug. 1935 .	Do.
Chak Dora (Shahpur) .	4 F . . .	15th Aug. 1935 .	Medium
Chak 109 R. B. . .	4 F . . .	5th Sept. 1935 .	Do.
Chak Branford (Lyallpur)	4 F . . .	10th Sept. 1935 .	Diseased
Narwala (Lyallpur) .	4 F . . .	20th Sept. 1935 .	Medium
Sharakpur . . .	4 F . . .	26th Sept. 1935 .	Diseased
Raewind . . .	4 F . . .	7th Oct. 1935 .	Do.
Chak 223 (Lyallpur) .	4 F . . .	24th Oct. 1935 .	Do.
Ganga-nagar (Jaranwala)	4 F . . .	5th Oct. 1935 .	Do.
Do. .	L. S. S. . .	Do. .	Do.
Chillianwala (Gujrat) .	L. S. S. . .	26th Sept. 1935 .	Medium
Do. .	38 F . . .	Do. .	Diseased
Do. .	45 F . . .	Do. .	Do.
Do. . .	4 F . . .	Do. .	Do.
Khanewal . . .	4 F . . .	25th Sept. 1935 .	Medium
Do. . . .	K. T. 23 . .	Do. .	Diseased
Do. . . .	K. T. 25 . .	13th Oct. 1935 .	Do.
Do. . . .	38 F . . .	Do. .	Do.
Multan . . .	43 F . . .	3rd Nov. 1935 .	Do.
	4 F . . .	Do. .	Do.
	38 F . . .	Do. .	Do.
	L. S. S. . .	Do. .	Do.
	73 F . . .	Do. .	Do.

Cottons from other parts of India

Place	Strain	Date of collection	Remarks
Sakrand (Sind)	4 F 98	10th Aug. 1935	Free
	289 F	Do.	Do.
	Do.	3rd Oct. 1935	Diseased
	4 F 98	Do.	Do.
	289 F	25th Sept. 1935	Do.
	Cambodia	14th Oct. 1935	Do.
	4 F 18 Parent	1st Nov. 1935	Do.
	4 F 18 × Meade Strain No. 94	Do.	Do.
	4 F 18 × Meade Strain No. 101	Do.	Do.
	4 F 18 × Meade Strain No. 27	Do.	Poor medium
	Meade parents	Do.	Diseased
	Sea Island × 285 F	Do.	Do.
	Do.	Do.	Do.
	185 F_2	Do.	Do.
	4 F 98	Do.	Do.
	A, 2 Bulk.2-45	Do.	Do.
	2-26-41	Do.	Do.
	Boss III 16	Do.	Do.
	Ashmoni 39	Do.	Do.
	C o II	Do.	Do.
	N. T. 21-8-8	Do.	Do.
	K. T. 25-81-5	Do.	Do.
	Desi 27 W.N.	Do.	Poor medium

After the paper was sent for publication many other strains of cotton have been examined with similar results.

Cottons from other parts of India—contd.

Place	Strain	Date of collection	Remarks
Surat	1027 A.L.F.	15th Aug. 1935	Medium
	Do.	7th Oct. 1935	Diseased
Nagpur	Buri	25th Oct. 1935	Free
	Verum	Do.	Do.
Indore	Malvi 1	29th Oct. 1935	Diseased
	Malvi 9	7th Sept. 1935	Free
	Do.	29th Oct. 1935	Diseased
	Cambodia	30th Oct. 1935	Do.
	Co II (Coimbatore)	Do.	Do.
	(Sanguineum × Sea Island) × $_2$U Upland	Do.	Medium
Dharwar	102 E	3rd Nov. 1935	Do.
	Gadag × 102 E	Do.	Do.
	Gadag 1	Do.	Do.
	Jaywant	Do.	Diseased
	Dharwar 1	Do.	Do.
Parbhani	American No. 1	8th Oct. 1935	Free
Ujjain	Cambodia	26th Oct. 1935	Do.

Indigenous cottons of the Punjab

Strain	Date of collection	Remarks
G. indicum var. *Mollisoni*	Throughout season	Free up to July and August. Then some plants medium, others free from disease.
G. indicum proper	26th Sept. 1935	Medium. Only cortex is affected. Medullary rays are all free.
G. neglectum proper	Do.	
G. neglectum var. *Rosea*	Do.	
G. sanguineum Var. *Major* (*i*) Broad-lobed	Do.	
(*ii*) Narrow-lobed	Do.	
Var. Minor (*i*) Broad-lobed	Do.	
(*ii*) Narrow-lobed	Do.	

Plants other than cotton examined for the disease

Plant	Date of collection	Remarks
Ocimum sanctum	8th Oct. 1935	Free
Vinca rosea	Do.	Do.
Hibiscus esculentus	15th Oct. 1935	Very slight tendency towards disease
	6th Nov. 1935	
H. rosasinensis	2nd Nov. 1935	Free
Abutilon indicum	17th Oct. 1935	Do.
	2nd Nov. 1935	Do.
Hollyhock and many others	1st Nov. 1935	Do.

II. Abnormalities in the roots

Many abnormal changes take place in the roots from the very seedling stage up to the time of maturity and in order to differentiate these changes from one another fresh material and sectioning are necessary. Only a very careful and prolonged study under the microscope has revealed the important and intricate changes which take place in the interior of the roots.

In the seedling stage the first symptom to be noticed is the brownian movement of the very small particles which give the appearance of small organisms (Plate XXX, fig. 4). They do not stop moving when treated with osmic acid and are not stained with iodine. These particles are seen to aggregate into cells and form a colony which is covered with a yellow mucilagenous mass (Plate XXX, fig. 4). These yellow masses can be easily distinguished under the low power of the microscope. When treated with an alkali, the yellow mass dissolves away but the particles still persist and exhibit brownian movement. These bodies when treated with nitric acid appear yellowish in colour. They are not soluble in strong nitric or hydrochloric acid. These yellow colonies become enlarged and assume a darker colour than before as the roots grow older. They are generally situated in the cortical region of the roots but are also found in the phloem. In a cross-section of the roots a number of them can be seen. The number continuously increases as the roots grow in thickness.

The second important change which takes place in the roots is the formation of some deposits in the cells of the cortex and the phloem (bark). These deposits are not seen in fresh sections but become apparent in stained preparations (Plate XXIX, fig. 4). They do not dissolve in alkalies or acids. They give all the protein reactions. They stain black with osmic acid. They are stained with all aniline stains like Methyl violet, Methylene blue, Giemsa and Lieshman's stains (Plate XXX, figs. 1 and 2).

These colourless deposits also gave all the colour reactions of tannins. About ten different colour reactions for tannins have been obtained with these deposits.

The number of cortical cells containing these deposits increases as the plant advances in age so much so that the whole tissue of the bark except the bast fibres becomes filled with it. The formation of these deposits then extends into the medullary rays and the xylem parenchymatous cells surrounding the vessels (Plate XXX, fig. 2). The vessels are free of these deposits except in some rare cases. Thus all the conducting channnels of the roots for the translocation of the elaborated foods and the storage places are filled with substances which appear to be a mixture of proteins, fats and tannins.

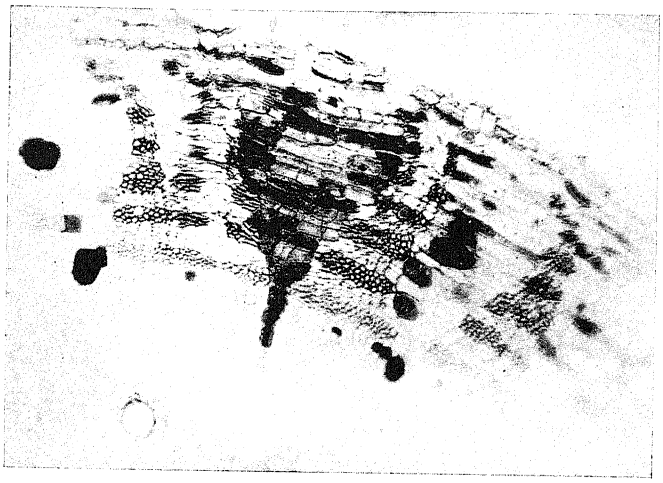

FIG. 1. Transverse section of root of 4-F American, showing the complex deposits in the cortical cells.

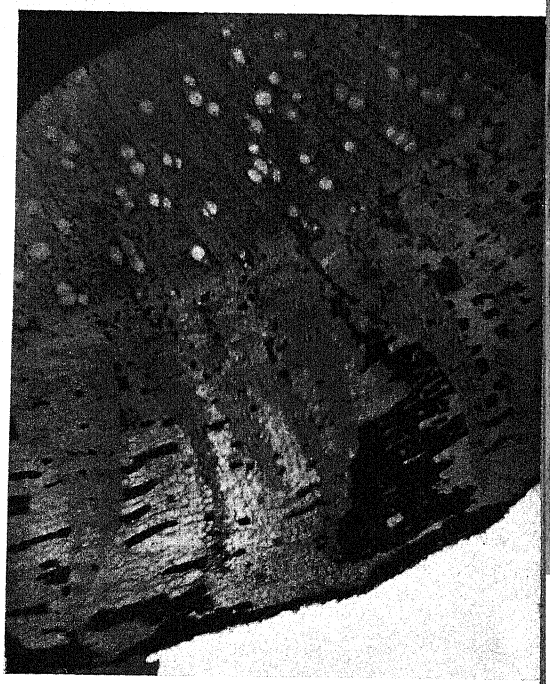

FIG. 2. Transverse section of root of 4-F America showing deposits in the cortical cells, phloe medullay rays, and xylem parenchyma.

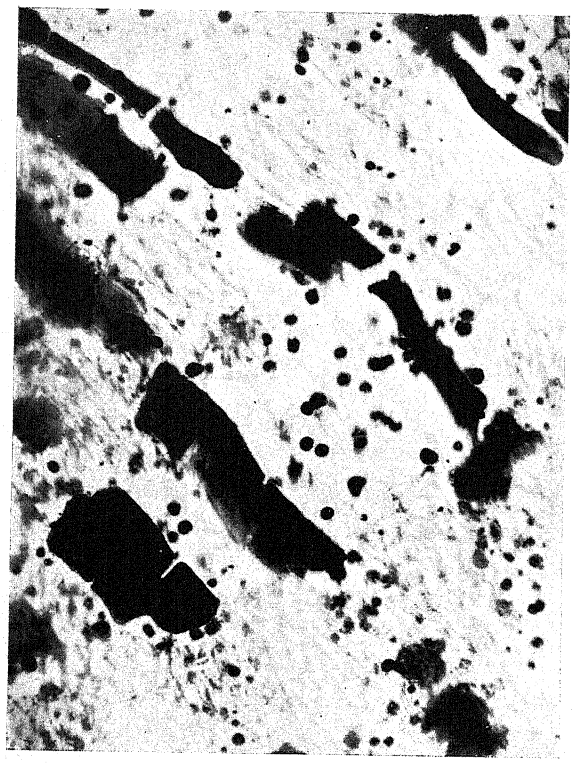

FIG. 3. Transverse section of root, showing the formation of the complex deposits.

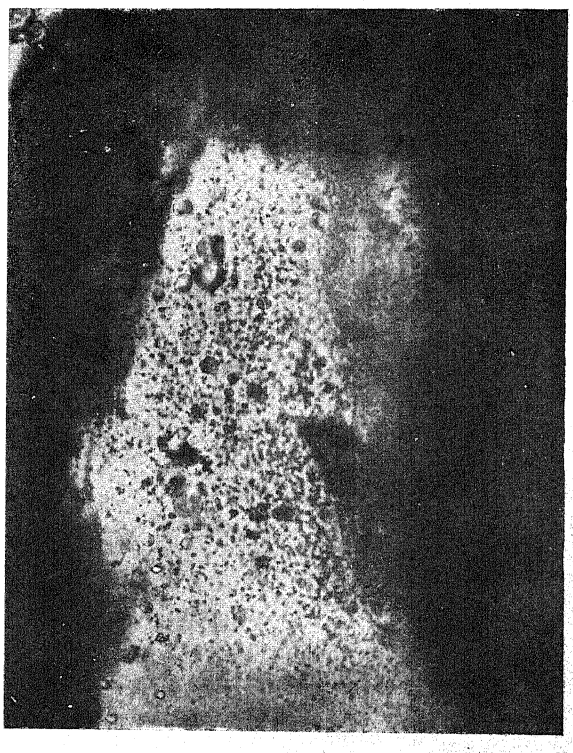

FIG. 4. A yellow colony with particles exhibiting brownian movement.

FIG. 1. Transverse section of root, showing semi-liquid deposits on the starch grains inside the cells of the root.

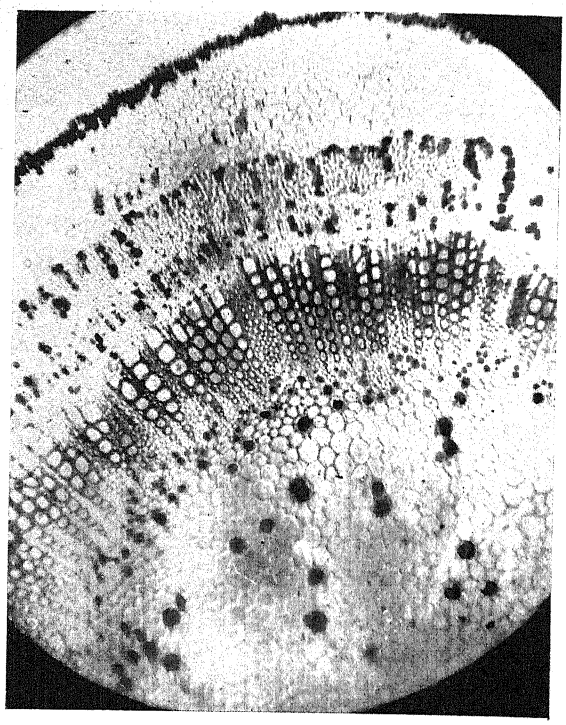

FIG. 2. Transverse section of stem of 4-F American, showing deposits in the epidermis, phloem, medullary rays and pith.

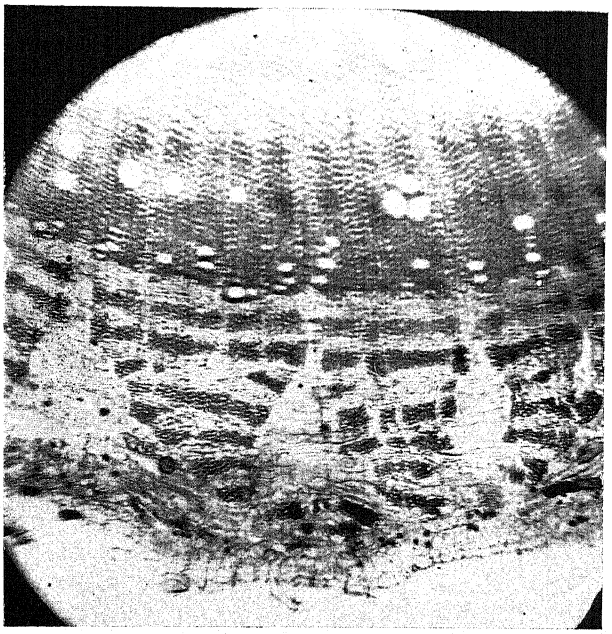

FIG. 3. Transverse section of roots of 4-F American, of a well-opened plant showing the absence of deposits.

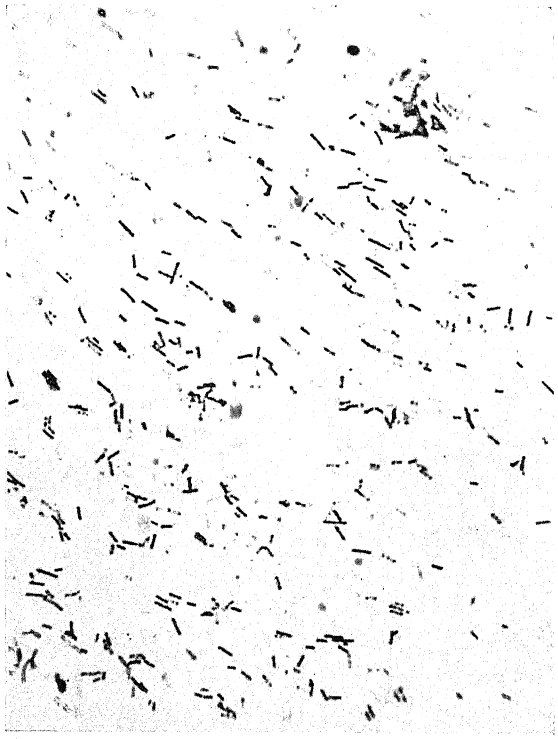

FIG. 4. The bacteria cultured from the embryo of 4-F American seed.

Similar deposits are also found in the lower portions of the stem (Plate XXXI, fig. 2) and the trouble extends upwards into primary and secondary branches. They can be seen in the topmost parts of the plant including the pericarp of the bolls. Sometimes the upper parts of the stem and branches are free while the lower parts of the same plant show these deposits.

These deposits found in the cells of the bark, the medullary rays, and the other cells are seen to develop from the particles exhibiting the brownian movement. These particles aggregate and form a small or a big colony which ultimately loses its granular character and appears as a semi-liquid mass. If the cell in which this process occurs contains starch grains, the fluid mass envelopes them (Plate XXX, fig. 3, and Plate XXXI, fig. 1). Later some reaction between the starch grains and the substance of the mass occurs and the starch grains lose their shape and character and a complex deposit described above is formed. This process occurs all along the storage and the food conducting cells of the plant.

In some cases the small particle surrounds the starch grain (Plate XXXI, fig. 1) and transforms the latter into a yellow body. Though the original shape of the starch grain is retained it does not stain with iodine. The transformed body of the starch is then stained with Methyl violet like the deposits described above, giving protein reactions.

Another abnormality found is the large accumulation of starch in the cortex, medullary rays, the xylem parenchyma and the pith. In many cases the roots and lower parts of the stems are completely filled with starch grains.

It was observed that, as in the case of the leaves, the time at which these abnormalities begin to appear in the bark of the roots and stems varies from plant to plant and filed to field. In some cases it was noticed in the month of July while in others in August or September or even later.

These abnormalities in the roots and stems were noticed only to a small extent or were entirely absent in plants which had well-opened bolls (Plate XXXI, fig. 3) while they were always present to a very great degree in badly-opened plants in the Punjab.

The following evidence indicates that the formation of these deposits in the roots and stems is an abnormal feature of the cotton plants here. (1) The deposits are not found ordinarily in plants either allied to cotton or plants belonging to other families. (2) It is possible to find cotton plants which do not show these abnormalities. The Verum and Buri cottons from the Nagpur Farm are free of these abnormalities even at harvest time. (3) 4-F cotton plants are also found in which these abnormalities are absent even at harvest time. (4) The period at which they appear varies from plant to plant. If it is a regular feature they should begin to appear at almost the same time at least in the same field in plants grown from seeds sown at the same time.

The great accumulation of starch at the fruiting stage in the roots and stems is also abnormal at the time when the starch should be hydrolysed and translocated to the fruiting parts. In plants whose bolls are opening well no such accumulation is noticed. In badly-opened plants the accumulation of starch is found to occur in roots, stems and branches.

The formation of these abnormal deposits is found in almost all varieties of cottons whether exotic or indigenous (Table I). It is most conspicuous in the Egyptian and American cottons grown in India. In *desi* cottons the formation of these deposits begins late in the season and is not so vigorous in most cases.

III. Presence of micro-organisms

Chloroplasts are stable bodies and though the green colouring matter adsorbed on their surface decompose, they are not ordinarily broken down to particles. The disintegration of chloroplasts in a healthy plant cannot occur except by the action of proteolylic enzymes secreted by micro-organisms present in the plants. Such disintegration of chloroplasts in leaves is reported by Sorokin [1927] and Eckerson [1926] in the case of tomato mosaic. It was, therefore, undertaken to determine whether any micro-organisms like bacteria or protozoa are present in the cotton plant.

Attemps were made in the first place to culture the organism from the leaves and it was found that rod-shaped bacteria in pure colonies developed in the culture media. These bacteria are different from *B. malvacearum* which causes the Black Arm disease of cotton. They have flagellae on all sides, while *B. malvacearum* has only one flagellum. Similar organisms were obtained from the root cultures.

Attempts were made to obtain bacterial cultures from the 4 F seed and it was found that the embryos in the seeds were infected, as the same form of bacteria developed from the seed cultures (Plate XXXI, fig. 4).

Later the seeds of the different strains of exotic and indigenous cottons were examined and these micro-organisms were found in each case. The cultures were made in duplicate.

These organisms have also been cultured from the old roots of cotton plants of the previous season and they are also found in abundance in the roots of *toria* which is a rotational crop with cotton in the Punjab.

Discussion

There are thus three principal types of abnormalities. (1) Disintegration of chloroplasts. (2) Formation of deposits in the bark and other conducting tracts with the accumulation of starch in diseased plants and (3) Presence of organisms in all parts of the plants.

It is well known that when pathogenic bacteria are present in a plant, changes of a chemical nature are produced by them in the host. They are known to produce injurious acids and toxic substances which inhibit physiological activities. On the other hand they also help the production of substances which stimulate the cell activities and either the multiplication or an increase in dimensions of the cells occurs.

In the cotton plant three effects of the presence of the pathogenes on the plant body are visible : (1) Hypertrophy of mesophyll cells, (2) Disintegration of chloroplasts and (3) Abnormal production of cell deposits. Even though the bacteria are present, the cotton plant offers great resistance to them and the plant does not succumb to them.

It may be tentatively concluded that the first two abnormalities result from the presence of bacteria in some form of their life-cycle though at present no definite demonstrative evidence to show that the organisms actually cause them* exists. The presence of organisms is now definitely established and cannot be disputed. It can be cultured in twenty-four hours from every part of the plant body. The novel feature is that when plant material is examined fresh, no living organisms are seen but they appear only after twenty-four hours when the sections of the root are left in sterilised water or kept in culture media*.

The chloroplasts of the leaves may suffer disintegration due to the toxins or proteolytic enzymes secreted by the bacteria. This has been verified by leaving a section of the leaf in hanging drop of water on a slide and infecting it with bacteria. By this means disintegration of plastids was obtained. This must be verified again as it was not possible to get a normal leaf free from this trouble and hence it cannot be said definitely whether the disintegration is actually caused by these organisms or not.

Secondary infection very probably occurs through the soil either from the ungerminated seeds, the old roots of the cotton plant of the previous season or from the roots of rotational crops especially the roots of *toria* which abound in these organisms. As a result of infection whether primary or secondary, pathological conditions result and these cell deposits are produced. It may be argued that the deposits produced in the cells may only be plastic material just like starch and are not produced as a result of patholocical condition. This appear unlikely as no such production of deposits occurs in cotton plants that grow normally and open well. They should also disappear when the bolls mature, but this does not happen. The cells contain them even after the plants have produced bolls.

* After this paper was sent for publication, the presence of the organism both inside the seed and the plant is definitely established It is also established that the complex cell deposits in the roots and stems are produced by the organism. A paper embodying these results and clarifying many points mentioned in this paper will soon be offered for publication.

These complex deposits remain in the cells as they are not translocated and also block up the normal channels of conduction.

The decomposition of chloroplasts in healthy leaves means a cessation of photosynthetic activity. The chloroplasts are the most important bodies in the life of a plant, as they are seats of all synthetic processes by which the food of the plant is manufactured. If they are gradually decomposed as the plant grows, less accumulation of food for the reproductive phase results.

In a normal plant all food, carbohydrates and proteins, which is manufactured by the leaves, is stored up in the storage tissues of the stems and roots. This accumulated food travels upwards in solution during the fruiting stage and is ultimately deposited in the seeds. The abnormal features in the roots and stems of the cotton plant described above do not permit of the translocation to the developing bolls of the food stored in these organs. The starch is also not hydrolysed and so cannot travel upwards. Most of it is used for the formation of complex deposits and so it is lost to the plant. Proteins are also precipitated and so are not available. Naturally those bolls which do not get proper nourishment do not develop well, its seeds remain empty and small and the lint has neither the required length nor strength. Whether a boll will open well or badly, depends upon the flow of nourishment to it. The number of well-opened bolls on a 'diseased' cotton plant will, therefore, depend on the time when the disease starts. If the trouble starts early in the season the whole plant fails badly.

The very wide variations in the yields of seed cotton from fifteen or twenty maunds to four or even two maunds per acre that are obtained in the Punjab on good soils and even in the same fields may now be more clearly understood than before on the basis of this internal trouble. These variations depend on the time of the incidence and the intensity of the 'disease'. It is also likely that other conditions of the soil and climate may have some effect on the intensity of the disease and time of its appearance in the plant. This will be investigated now.

Red-leaf disease

The reddening of the leaves of American cottons was first reported by Butler [1908] and then by Burt and Nizamuddin [1919]. Since then the term Red-leaf disease has come into use. In view of what is stated above, the reddening of the leaf is due to secondary changes in the leaf after it has ceased functioning. The reddening may occur at the normal cessation of the functional activity of the leaves. The premature reddening of the leaves is caused by the premature death of the leaves and for this reason such reddening of the leaves may be regarded as the external symptom of the internal disease. But it is also noticed that reddening of the leaves may not occur at all even though the plant is diseased and opens badly. Innumerable American cotton plants which have green leaves but which

are diseased and have opened badly have been observed. Reddening of the leaves should not be regarded as a true indicator of the internal trouble. A plant with reddened leaves may open better than a plant with green leaves if the reddening is of the normal character. The term Red-leaf disease in connection with the 'disease ' under investigation should now be discontinued. In indigenous cottons, the reddening of the leaves does not occur in any marked degree even though the same trouble exists there.

It is clear from the results of these investigations that the abnormalities described above are not confined to the exotic cottons but are found in indigenous cottons as well. As no bad opening is noticed in indigenous cottons, one might be led to infer that these abnormalities are not the cause of the bad opening in American cottons. In the case of *desi* cottons the trouble starts later in the season and conduction of food substances is not as much impeded as in the case of American cottons. No accumulation of starch in the plant is, therefore, noticed in the former. As sufficient nourishment is available for the remaining bolls, no bad opening occurs. In the case of American cottons sufficient nourishment is not available for all the bolls that remain on the plant and bad opening takes place, its extent depending on the time of incidence and intensity of the disease. It may be regarded as a fortunate occurrence that American cottons showed these external symptoms of premature reddening of the leaves and bad opening of the bolls, otherwise this internal trouble would have been left still undiscovered.

Conclusions

It is safe to conclude that these abnormalities in the American cotton are the cause of the bad opening of the bolls through want of nourishment. Though bad opening of bolls occurs in a greater or less degree in different years, the bad opening of bolls is always present. The cotton crop in the Punjab in the present season is reported to be normal but there is not a single field, as far as has been observed, which is free from plants showing bad opening of the bolls. In some fields the proportion of badly-opened bolls is very high and includes the whole crop ; in others it is low.

These badly-opened plants in most cases are neither small nor undersized so far as their vegetative growth is concerned and, therefore, the question of the deficiency of nitrogen in the soil does not arise. It is also noticed that a very small plant much below normal has very well-opened bolls if it is not diseased, while a tall and vigorous plant may have all its bolls badly-opened. Bolls smaller in size than the average, have been observed to open well if the proper nourishment is available, whereas large bolls in ' diseased ' plants crack and open badly.

The yield of seed cotton in American and indigenous cottons is less than it would be if these abnormalities of the leaves and roots did not exist. It is possible that the low yields in India as compared to other countries may be partly due to this internal trouble.

Summary

Micro-chemical study of American cottons grown in the Punjab and other places in India has revealed some very abnormal features, not noticed before.

Disintegration of the chloroplasts, in green and healthy-looking leaves first begins to occur. The time when it first begins appears to vary from plant to plant. This is followed by an accumulation of starch in the mesophyll cells. These cells also suffer elongation in different planes and this gives rise to crumpling, curling and thickening of the leaves. Later, the cells become filled with yellowish deposits which give reactions of tannins, fats and proteins. Development of anthocyanins and anthoxanthins occur in the epidermal cells and produces reddening of the leaves.

In the cortex of the roots small, yellow masses first appear. Each yellow mass contains minute bodies which exhibit rapid brownian movement. Such minute bodies have also been noticed in the cotyledons of the seed and the foliage leaves. These bodies aggregate in the cortical, phloem and other conducting parenchymatous cells and first form small or large colonies which later lose their granular appearance, and become semi-liquid in nature. These bodies react with the starch grains in the cells of the cortex, pith medullary rays and xylem parenchyma and ultimately form complex deposits of substances which give the reactions of proteins, fats and tannins. Such deposits are produced in all the cells, except the xylem vessels of the roots, stems and branches.

Micro-organisms in the form of bacteria have been cultured from either the leaf, stem, root and the embryo of the seed of the plant. They are different from the organism that causes the Black-arm disease of cotton.

These abnormalities are found in more than fifty different strains of cottons, exotic or indigenous so far examined. There are also a few strains which are free of them.

In well-opened American plants the abnormalities in the roots and stems are absent.

It is tentatively put forward that the presence of the micro-organism, in some stage in its life-cycle, is responsible for the destruction of the chloroplasts and consequent inhibition of the food-manufacturing processes of the plant. Whatever food that is stored in the roots and stems of the plant during its vegetative period, is mostly used in the formation of the complex deposits in the storage and conducting tissues and the remaining portion is not able to translocate rapidly to the developing bolls in the fruiting stage on account of the blocking up of the conducting elements by the same deposits. The bolls thus suffer want of nourishment, crack prematurely and contain immature seeds with poor lint. The number of well-opened bolls on the plant and consequently its yield depend on the time the ' trouble ' starts and its intensity.

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DEVELOPMENTAL VARIATION IN THE PADDY GRAIN

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CONTENTS

		PAGE.
I.	INTRODUCTION	396
II.	METHOD OF GRAIN MEASUREMENT	397
III.	SIZE OF SAMPLE	397
IV.	VARIATION WITHIN A PANICLE	401
V.	VARIATION BETWEEN PANICLES OF ONE PLANT	403
VI.	DIFFERENCE IN VARIABILITY BETWEEN DIFFERENT STRAINS OF THE SAME CLASS	408
VII.	DIFFERENCE IN VARIABILITY BETWEEN PURE LINES OF DIFFERENT CLASSES	410
VIII.	EFFECT OF SPACING ON GRAIN SIZE AND VARIABILITY	412
IX.	EFFECT OF HARVESTING DATES ON GRAIN SIZE.	414
X.	EFFECT OF SEASON ON GRAIN WEIGHT	416
XI.	EFFECT OF SEASON ON GRAIN LENGTH	418
XII.	EFFECT OF MANURE ON GRAIN WEIGHT	419
XIII.	EFFECT OF MANURE ON GRAIN SIZE	444
XIV.	EFFECT OF MANURE ON THE DIFFERENT PARTS OF THE PADDY GRAIN	448
XV.	DIFFERENCE IN GRAIN SIZE DUE TO LOCALITY	451
XVI.	DISCUSSION	456
XVII.	SUMMARY	458
	REFERENCE	459

I. INTRODUCTION

The classification of paddy on grain dimensions has engaged the attention of various workers in recent years. Beale [1927] and Sethi [1930] found that the grain-size characters are the least influenced by environment, and they therefore based their system of classification on the grain dimensions. In addition

to the advantage of the stability of the grain dimensions classification based on these corresponds largely with commercial grading. Recently Ramiah [1933] found that grain size is to some extent affected by seasonal variations.

The dimensional characters of the grain have also been utilised in determining the purity of strains, as according to Ramiah [1933] these do not vary to any appreciable extent within a pure line. This is not in accordance with the statements of van der Stok [1912], who found that even within a pure line size and weight of the grains depend upon the size of the panicle and the fertility of the soil. Although the variations which occur will never be of sufficient magnitude to invalidate a system of classification based thereon, there has not hitherto appeared any reliable study of the magnitude of these variations and of the degree of variability which may be expected under different conditions. Further, in the study of the inharitence of grain-size characters a knowledge of how grain dimensions may vary in a non-inherited manner is desirable.

II. Method of grain measurement

The measurement of grains by a screw gauge is a tedious and clumsy method. Throughout this investigation all measurements have been made with a special seed-measuring apparatus supplied by Messrs. Gallenkamp & Co., Ltd., London. This instrument is graduated in centimeters and millimeters and reads direct on a dial to 0·1 mm. and by estimation to 0·05 mm. When suitably held in a retort-stand clamp it is possible to carry out measurements accurately and rapidly. Unless the statistical examination of the results requires it, the individual readings are not written down. When a frequency distribution suffices, a series of small beakers each marked with its appropriate class interval is arranged on the bench and as each grain is measured it is put into the appropriate beaker. When the required number of grains has been measured the number in each beaker gives the frequencies in each class.

III. Size of sample

Ramiah differs in opinion from Lord [1929] as regards the size of sample required to give a reliable result. The latter concluded that a minimum of twenty grains was necessary, while Ramiah who has restricted his measurement of the sample to the top and middle portion of the panicle has found a six-grain sample satisfactory for inheritance studies. But in the present investigation it has been found that to be representative a random sample must consist of at least 100 grains.

Five hundred grains of the pure line *Kabaing Taungdeikpan* B 30-104 were taken at random and measured and means, standard deviations, etc., for grain length were determined separately for different sizes of sample. The results are given in Table I.

TABLE I

(a) Twenty-five-grain sample

No.	Mean (mm.)	S. E. of mean	Variance of mean	S. E. of mean of all samples	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$ (mean square)
1	8·520	0·049929	0·0024928		24	1·4360	0·05983	0·8945
2	8·576	0·070000	0·0049000		24	2·8224	0·11760	1·2323
3	8·584	0·064949	0·0042183		24	2·4298	0·10124	1·1574
4	8·536	0·068520	0·0046950		24	2·7043	0·11268	1·2110
5	8·592	0·053647	0·0028780					
6	8·424	0·101370	0·0102760					
7	8·582	0·062724	0·0039345					
8	8·536	0·092384	0·0085350					
9	8·560	0·067840	0·0046023	0·07287				
10	8·584	0·069630	0·0048484					
11	8·360	0·079968	0·0063948					
12	8·432	0·058454	0·0034070					
13	8·552	0·070876	0·0050234					
14	8·400	0·060883	0·0037068					
15	8·480	0·076630	0·0058721					
16	8·528	0·082240	0·0067634					
17	8·536	0·072160	0·0052071					
18	8·504	0·079260	0·0062820					
19	8·504	0·067960	0·0046185					
20	8·456	0·047300	0·0022373					

(*b*) *Fifty-grain sample*

No.	Mean (mm.)	S. E. of mean	Variance of mean	S. E. of mean of all samples	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$ (mean square)
1	8·548	0·042883	0·0018389		49	4·4153	0·09011	1·0993
2	8·560	0·047637	0·0022693		49	5·4487	0·10982	1·1981
3	8·412	0·050384	0·0025389		49	6·0961	0·12441	1·2588
4	8·540	0·055635	0·0030953	0·051045	49	7·4320	0·15167	1·3596
5	8·582	0·048638	0·0023656					
6	8·386	0·051330	0·0026346					
7	8·476	0·047434	0·0022499					
8	8·504	0·054601	0·0026570					
9	8·520	0·045579	0·0020774					
10	8·480	0·041536	0·0017252					

(*c*) *Hundred-grain sample*

No.	Mean (mm.)	S. E. of mean	Variance of mean	S. E. of mean of all samples	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$ (mean square)
1	8·554	0·030580	0·0009351		99	9·1630	0·09255	1·1147
2	8·526	0·040920	0·0016744		99	16·4110	0·16577	1·4041
3	8·484	0·035482	0·0012589	0·039582	99	12·0590	0·12180	1·2500
4	8·490	0·036134	0·0013056		99	12·7930	0·12926	1·2785
5	8·500	0·033060	0·0010930					
	8·510	0·016055	0·0002578		499	64·4200	0·12909	1·2791

Mean of 500 grains . . . . . =8·510
Standard error of mean . . . . . =0·016055
Variance of mean . . . . . =0·0002578

From the above tables it can be seen that in many cases of the twenty-five grain and fifty-grain samples the differences of the means are statistically significant but in the hundred-grain samples no two means differ from each other with certainty. Further, the general mean of 500 grains being 8·510 mm. and the S. E. 0·016, a representative sample should have a mean lying between the limits 8·478 and 8·542 mm. Out of twenty twenty-five grain samples twelve have means outside these limits ; out of ten fifty-grains samples seven have their means outside these limits, but only in one case is a hundred-grain sample outside the limits. On these grounds it is considered that a sample of hundred grains can always be relied on to give a representative reading and were it not for practical difficulties larger samples would appear desirable.

The extent of variability is an important character in grain, because uniformity in grain size conduces to lower breakage in milling and a smaller portion of unhulled grains passing the huller. When the variability between the first four samples of each size in Table I is compared, it is seen that although variability does not differ significantly between samples of the same size, yet they differ in two cases between samples of one size and another.

TABLE II

*Values of S. D. of z**

(*a*) *Within the groups*

	Within 25-grain sample	Within 50-grain sample	Within 100-grain sample
$\sqrt{\frac{1}{n}}$ . .	0·2040	0·1428	0·1005
$\sqrt{\frac{1}{n}}$ . .	0·4080	0·2856	0·2010

* The validity of distribution of z for the comparison of several groups is here assumed to be for a random sample from a homogenous population.

(b) Between the groups

	Between 1st and 2nd groups	Between 1st and 3rd groups	Between 2nd and 3rd groups	Between total and first group	Between total and second group	Between total and third group
$\sqrt{\frac{1}{-}(\frac{1}{n_1}+\frac{1}{n_2})}$	0·1761	0·1577	0·1235	0·1476	0·1058	0·07778
$\sqrt[2]{\frac{1}{2}(\frac{1}{n_1}+\frac{1}{n_2})}$	0·3522	0·3154	0·2470	0·2952	0·2116	0·15556

IV. Variation within a panicle

Six panicles of the same plant of the pure line *Ngasein C2104* were roughly divided into three parts of equal lengths and the frequency distributions of the grain lengths for each part, top, middle and bottom, are given in Table III.

Table III

Frequency distributions of portions of panicles of Ngasein 2104

Classes	Panicle No. (*a*)			Panicle No. (*b*)			Panicle No. (*c*)			Panicle No. (*d*)			Panicle No. (*e*)			Panicle No. (*f*)		
	T	M	B	T	M	B	T	M	B	T	M	B	T	M	B	T	M	B
Unfilled grains . .	3	3	14	2	11	11	0	15	27	1	8	7	3	7	10	1	8	22
7.2 . . .	0	2	3															
7.4 . . .	0	3	2		1	1												1
7.6 . . .	1	1	4		2	1		1			1	2						1
7.8 . . .	3	3	5			1		3	1	1	3	1	1					2
8.0 . . .	4	6	9	3	2	1	2	2	7	5	6	2		2	5	1	1	3
8.2 . . .	10	6	4	2	9	5	6	8	6	4	2	4	3	1	3	4	1	2
8.4 . . .	9	10	7	9	6	6	8	12	13	10	6	10	6	5	5	4	5	2
8.6 . . .	15	11	10	11	7	6	8	10	11	7	11	8	4	5	10	4	5	7
8.8 . . .	11	6	5	8	7	6	8	7	5	5	1	1	6	4	8	7	3	3
9.0 . . .	3	1	1	5	2	1	5	3		1			2	2	1	4	5	2
9.2 . . .																	1	
Total .	56	49	48	38	36	28	37	46	43	33	30	28	22	19	32	24	21	23

The results are summarised in Table IV and the values of t and P [Fisher, 1925] are given in Table V.

TABLE IV

Ngasein 2104 *grain lengths of top, middle and bottom portions of panicles*

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	Mean
Top . . .	8·454	8·579	8·557	8·418	8·545	8·600	8·526
Middle . . .	8·267	8·406	8·435	8·307	8·547	8·657	8·436
Bottom . .	8·208	8·414	8·391	8·336	8·502	8·365	8·369

TABLE V

Ngasein 2104, *values of* t *and* P *for differences of means*

Values	Top and middle	Top and bottom	Middle and bottom
t	1·4980	3·4438	1·0252
P	More than 0·1	Less than 0·01	More than 0·2

There is a significant difference in grain lengths between the top and bottom portions. Though the means in other cases do not differ significantly, it appears probable that a relationship exists between position on the panicle and the length of the grains, as the latter decreases towards the base of the panicle.

It has been stated by Ramiah and Parthasarathi [1933] and by van der Stok [1912] that the lower portion of the panicle bears a higher proportion of empty grains and this has been confirmed. The latter also states that the mean grain weight decreases downwards on the panicle and this has also been confirmed as the results given in Table VI show.

TABLE VI

10 × mean weight of grains in grams from different portions of six panicles, Ngasein 2104

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	Mean
Top . . .	2·8006	2·8120	2·7154	2·6862	2·6792	2·7675	2·7434
Middle . . .	2·6507	2·6628	2·6943	2·6286	2·6570	2·7046	2·6663
Bottom . .	2·5472	2·5738	2·5943	2·5985	2·5790	2·5790	2·5786

The values of *t* and *P* obtained are given in Table VII.

TABLE VII

Values of t *and* P

Values	Top and middle	Top and bottom	Middle and bottom
	2·9050	6·6359	6·3736
P	Between 0·02-0.01	Less than 0·01	Less than 0·01

V. VARIATION BETWEEN PANICLES OF ONE PLANT

Four plants of two local varieties, *Ngasein 2104* and *Theikpan Taungdeikpan* planted at one ft. × one ft. spacing were chosen at random and the panicles were threshed separately, the grains counted and their lengths measured. The number of panicles in each plant, being dependent on the cultural conditions, varies greatly. Means* and variance of means were calculated from individual grains separately for each panicle and for each plant and are given in Tables VIII to XIII.

* The method of analysis by compartments has been used in this chapter. The calculations of S. E. and variance ot means were done separately for individual panicle and individual plants from individual grains.

TABLE

Ngasein 2104, *mean grain lengths of separate panicles, mean variances and standard error*

Penicle No.	Plant No. 1			Plant No. 2			Plant No. 3			Plant No. 4		
	Mean (mm.)	Variance of mean	S. E. of mean of all panicles	Mean (mm.)	Variance of mean	S. E. of mean of all panicles	Mean (mm.)	Variance of mean	S. E. of mean of all panicles	Mean (mm.)	Variance of mean	S. E. of mean of all panicles
a	8·3175	0·001093		8·4955	0·0007401		8·2212	0·0007517		8·5364	0·0005982	
b	8·4726	0·001169		8·4461	0·0005235		8·3000	0·0008724		8·6704	0·0008276	
c	8·4555	0·000695		8·5213	0·0006983		·48 0	0·0011225		8·4487	0·0006044	
d	8·6438	0·001125	0·036945	8·4632	0·0007236	0·030183	8·4943	0·0009467	0·034200	8·6603	0·0007862	0·030120
e	8·5225	0·001087		8·4259	0·0006334		8·4177	0·0008876		8·3978	0·0007624	
f	8·3502	0·000994		8·4392	0·0007212		8·2065	0·0014980		8·5807	0·0008974	
g	8·5382	0·002092		8·3562	0·0007589		8·4600	0·0009494		8·5469	0·0006314	
h				8·5733	0·0009676					8·4852	0·0008937	
i				8·4863	0·0007669					8·6696	0·0012588	
j				8·3062	0·0013340							
k				8·4598	0·0006287							
l				8·5625	9·0011337							
m				8·4865	9·0014024							

TABLE IX

Ngasein 2104, *mean grain lengths of separate plants*

Plant No.	Mean mm.	S. E. of mean	Variance of mean	Remarks
1	8·4375	0·01346	0·0001810	Any panicle mean outside the limits 8·4106 and 8·4644 shows significant aberration.
2	8·4394	0·00790	0·0000624	Any panicle mean outside the limits 8·4236 and 8·4443 shows significant aberration.
3	8·3540	0·01226	0·0001504	Any panicle mean outside the limits 8·3295 and 8·3785 shows significant aberration.
4	8·5384	0·00995	0·0000991	Any panicle mean outside the limits 8·5181 and 8·5579 shows significant aberration.
Total of four plants	8·4424	0·012817		Any plant mean outside the limits 8·4168 and 8·4680 shows significant aberration.

TABLE X

Ngasein 2104, *mean difference and S. E. differences are given in mm.*

Ngasein 2104	Plant No. 2	Plant No. 3	Plant No. 4
Plant No. 1. .	0·0019 ± 0·0015602	0·0835 ± 0·018204	0·1009 ± 0·016736
Plant No. 2. .		0·0854 ± 0·014590	0·0990 ± 0·012710
Plant No. 3. .			0·1844 ± 0·015790

TABLE XI

Theikpan Taungdeikpan, *mean grain lengths of separate panicles, mean variances and standard error*

Panicle No.	Plant No. 1			Plant No. 2			Plant No. 3			Plant No. 4		
	Mean (mm.)	Variance of mean	S. E. of mean of all panicles	Mean (mm.)	Variance of mean	S. E. of mean of all panicles	Mean (mm.)	Variance of mean	S. E. of mean of all panicles	Mean (mm.)	Variance of mean	S. E. of mean of all panicles
a . .	8·647	0·000545		8 619	0·000390		8·620	0·000577		8·605	0·000489	
b . .	8.577	0·000892		8·550	0·000643		8·776	0·000648		8·522	0·000530	
c . .	8·749	0·001107		8·675	0·000921		8·672	0·000473		8·544	0·000596	
d . .	8·525	0·000699		8·729	0·000874		8·684	0·000559		8·691	0·000595	
e . .	8·781	0·000749		8·622	0·000491		8·692	0·000771		8·736	0·000580	
f . .	8·764	0·000633		8·779	0·000739		8·634	0·000749		8·553	0·000649	
g . .	8·727	0·000608		8·758	0·000835		8·533	0·001608		8·691	0·001312	
h . .	8·543	0·000942	0·03100	8·674	0·000926	0·03170	8·697	0·001581	0·03080	8·609	0·001045	0·03030
i . .	8·816	0·000882		8·664	0·000565		8·759	0·000745		8·658	0·000881	
j . .	8·779	0·001124		8·529	0·001043		8·736	0·000570		8·514	0·000693	
k . .	8·774	0·000988		8·583	0·001071		8·645	0·000745		8·503	0·000617	
l . .	8·678	0·000578		8·561	0·001216		8·644	0·001068		8·581	0·001056	
m . .	8·566	0·000617		8·563	0·002357		8·729	0·001366		8·630	0·000859	
n . .	8·784	0·001097								8·600	0·001903	
o . .	8·769	0·001988								8·560	0·001037	
p . .										8·543	0·000953	

TABLE XII

Theikpan Taungdeikpan, *mean grain length of separate plants*

Plant No.	Mean (mm).	S. E. of mean	Variance of mean	Remarks
1	8·7010	0·008056	0·0000649	Any panicle mean outside the limits 8·6849 and 8·7171 shows significant aberration.
2	8·6499	0·008013	0·0000642	Any panicle mean outside the limits 8·6339 and 8·6659 shows significant aberration.
3	8·6859	0·007702	0·0000593	Any panicle mean outside the limits 8·6704 and 8·7014 shows significant aberration.
4	8·5976	0·007243	0·0000525	Any panicle mean outside the limits 8·5832 and 8·6120 shows significant aberration.
Total of four plants	8·6586	0·010304		Any plant mean outside the limits 8·6380 and 8·6792 shows significant aberration.

TABLE XIII

Theikpan Taungdeikpan, *mean difference and S. E. differences are given in mm.*

Theikpan Taungdeikpan	Plant No. 2	Plant No. 3	Plant No. 4
Plant No. 1 .	0·05114±0·011363	0·01508±0·011146	0·10337±0·010834
Plant No. 2 .		0·03605±0·011110	0·05224±0·010802
Plant No. 3 .			0·08829±0·010573

From Tables VIII to XIII it appears that in both varieties the lengths of the grains of different panicles of one plant differ from one another significantly. In all cases more than half of the comparisons shown significant results. Between panicles of different plants there are also, as would be expected, significant differences between means. Moreover when each of the panicles is compared with its respective plant mean they show in most cases significant differences and most of the means of the panicles lay outside the limits of their respective plant means.

Comparisons between plants of the same class also show significant differences between the means. This holds good for both varieties and at both one ft. × one ft. and two ft. × two ft. spacings. This is shown in Tables XIV and XV.

TABLE XIV

Comparison of grain lengths of separate plants of Ngasein 2104 *and* Theikpan Taungdeikpan (*Spacing 2 ft. × 2 ft.*)

Plant No.	Ngasein				Theikpan Taungdeikpan			
	Mean (mm.)	S. E. of mean	Variance of means	S. E. diff. of the mean of 4 plants	Mean	S. E. of mean	Variance of mean	S. E. diff. of the mean of 4 plants
1	8·428	0·005576	0.0000311	0·00748	8·609	0·005323	0·0000283	0·00648
2	8·341	0·006597	0.0000534		8·474	0·005462	0·0000298	
3	8·458	0·006631	0.0000440		8·541	0·006748	0·0000425	
4	8·515	0·006348	0.0000403		8·593	9·005069	0·0000257	

TABLE XV

Final results showing mean differences and corresponding S. E. differences

Plant No.	Ngasein 2104			Theikpan Taungdeikpan		
	2	3	4	2	3	4
1	0·087±0·00864	0·030±0·00866	0·087±0·00845	0·185±0.00762	0·068±0·00859	0·016±0·00735
2		0·117±0·00935	0·174±0·00915		0·067±0·00868	0·119±0·00745
3			0·057±0·00918			0·052±0·00844

VI. DIFFERENCE IN VARIABILITY BETWEEN DIFFERENT STRAINS SELECTED FROM THE SAME CLASS

Six strains of *Ngasein* (C class) and ten strains of *Kabaing Taungdeikpan* (B class) selected in 1929 from cultivators' varieties and grown for the first time in 1930-31, were chosen for the experiment. From the measurements of length the analyses were done separately and are given in Tables XVI (*a*) and XVI (*b*).

TABLE XVI (*a*)

Variation of grain length of Ngasein (*six strains*)

Strain No.	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Log$_e$ (mean square)	$\frac{1}{n}$
30—24 . .	499	60·900	0·1220	1·2502	0·002004
30—25 . .	449	62·657	0·1256	1·2643	0·002004
30—26 . .	499	100·350	0·2011	1·4998	0·002004
30—27 . .	499	63·857	0·1280	1·2741	0·002004
30—28 . .	499	50·374	0·1009	1·1558	0·002004
30—31 . .	499	62·990	0·1262	1·2703	0·002004

Standard deviation of z is 0·0447.

TABLE XVI (*b*)

Variation of grain length of Kabaing Taungdeikpan (*ten strains*)

Strain No.	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Log$_e$ (mean square)	$\frac{1}{n}$
30—104 . .	499	64·950	0·13016	1·2829	0·002004
30—105 . .	499	74·808	0·14992	1·2829	0·002004
30—106 . .	499	68·652	0·13758	1·4003	0·002004
30—107 . .	499	79·176	0·15867	1·3831	0·002004
30—108 . .	499	78·172	0·15666	1·3771	0·002004
30—113 . .	499	67·290	0·13485	1·3007	0·002004
30—119 . .	499	71·366	0·14302	1·3314	0·002004
30—120 . .	499	71·366	0·14302	1·3314	0·002004
30—121 . .	499	75·602	0·15151	1·3594	0·002004
30—123 . .	499	82·092	0·16451	1·4340	0·002004

Standard deviation of z is 0·0447

Ngasein 30-26 is definitely different in variability from other srains, and *Ngasein 30-28* gives a rather high value of z in comparison with the others. Except in one case the value of z fails to pass the required point of significance. In *Taungdeikpan* also the differences in variability are significant in several cases. Therefore variability is not constant even with one type or class. This type of variability is of importance because uniformity of grain size reduces breakage in milling.

VII. Difference in variability between pure lines of different classes

The sum of squares, mean squares, etc., of four plants each of *Ngasein C2104* and *Theikpan Taungdeikpan* (B class) grown at one ft. × one ft. spacing were calculated separately and are compared in Table XVII.

Table XVII

Variation of grain lengths in different classes of pure line paddies

Plant No.		Degrees of freedom	Sum of squares	Mean squares	$\frac{1}{2}\text{Log}_e$ (mean square)	$\frac{1}{n}$
C2104 Ngasein	1	644	74·236	0·11527	1·2224	0·0015528
	2	1390	120·720	0·08685	1·0807	0·0007194
	3	658	65·308	0·09925	1·1475	0·0015198
	4	767	58·418	0·07616	1·0152	0·0013038
Total	.	3462	337·970	0·09762	1·1392	0·0002889
Theikpan Taungdeikpan.	1	1585	163·210	0·10297	1·1660	0·0006309
	2	1507	146·040	0·09691	1·1355	0·0006636
	3	1817	196·050	0·10790	1·1896	0·0005504
	4	1769	154·330	0·09289	1·1144	0·0005653
Total	.	6681	681·220	0.10196	1·1610	0·0001497

Though the differences in variability between plants are large they cannot be judged significant on these data. The variability does not depend on the type of grain, any two types may or may not differ in variability. In *Ngasein* and *Taungdeikpan* the variability of one plant may differ significantly from the variability of another plant of the same strain, while the two types as a whole do not differ significantly. The results are summarised in Table XVIII. In Table XVIII significant variations are *italicised*.

TABLE XVIII

Differences in variability, Ngasein *and* Taungdeikpan

		Ngasein C 2104			*Taungdeikpan* (B Class)			
		2	3	4	1	2	3	4
Ngasein	1	*0·1484 ±0.0337*	*0·0816±0.0382*	*0·2140±0.0378*	0·0631±0·0320	*0·0836±0·0333*	0·0395±0·0324	*0·1047±0.0333*
	2		*0·0668±0.0334*	*0·0656±0.0318*	*0·0853±0·0259*	*0·0548±0·0263*	*0·1089±0·0252*	0·0337±0·0253
	3			*0·1324±0.0376*	0·0185±0·0328	0·0120±0·0330	0·0421±0·0312	0·0331±0·0313
	4				0·0509±0·0311	0·0204±0·0313	*0·0745±0·0304*	0·0007±0·0306
Taungdeikpan	1					0·0305±0·0255	0·0236±0·0243	*0·0516±0·0244*
	2						*0·0541±0·0246*	0·0211±0·0277
	3							*0·0652±0·0236*

From these data it appears that variability between plants can be held to be significant, but differences in variability between the two classes of paddy are not reliable. Variability may, therefore, agree between quite different classes of paddy and may differ between strains of the same class. Since variation in grain size will affect milling behaviour, it is important that in selection work the degree of variability to which a strain is liable should not be overlooked.

VIII. Effect of spacing on grain size and variability

The effect of spacing on the two varieties, *Ngasein* and *Taungdeikpan*, is shown in Tables XIX, XX, XXI and XXII. The means, variance of mean and standard errors were calculated separately for four plants of each variety at one ft. × one ft. and two ft. × two ft. spacings.

Table XIX

Ngasein, *mean length differences and standard error (2 ft. × 2 ft. spacing and one ft. × one ft. spacing)*

	Plant No.	*Ngasein 2104* 2 ft. × 2 ft.			
		1	2	3	4
Ngasein 1 ft. × 1 ft.	1	0·010±0·0145	*0·097±0·0149*	0·020±0·0150	*0·077±0.0148*
	2	0·011±0·0096	*0·098±0·0102*	0·019±0·0103	*0·076±0.0101*
	3	*0·074±0·0134*	0·013±0·0139	*0·104±0·0140*	*0·159±0.0138*
	4	*0·110±0·0114*	*0·197±0·0119*	*0·080±0·0120*	*0·023±0.0118*

Table XX

Theikpan Taungdeikpan, *mean length differences and S. E. (2 ft. × 2 ft. spacing and 1 ft. × 1 ft. spacing)*

	Plant No.	*Theikpan Taungdeikpan* 2 ft. × 2 ft.			
		1	2	3	4
Theikpan Taungdeikpan 1 ft. × 1 ft.	1	*0·092±0.0097*	*0·227±0·0097*	*0·163±0·0105*	*0·111±0.0095*
	2	*0·041±0.0096*	*0·176±0·0097*	*0·112±0·0104*	*0·060±0.0094*
	3	*0·077±0.0094*	*0·212±0·0094*	*0·145±0·0102*	*0·096±0.0092*
	4	0·011±0.0090	*0·124±0·0091*	*0·060±0·0098*	0·008±0.0088

Mean differences are correct to three places of decimals. Those printed in *italics* are significant.

TABLE XXI

Ngasein *and* Taungdeikpan, *mean lengths, mean variances and standard error for 1 ft. × 1 ft. and 2 ft. × 2 ft. spacings*

Variety	*Ngasein*		*Theikpan Taungdeikpan*	
Spacing . .	1 ft. × 1 ft.	2 ft. × 2 ft.	1 ft. × 1 ft.	2 ft. × 2 ft.
Mean . . .	8·4424	8·4355	8·6586	8·5542
S. E. of mean .	±.005309	±0·00329	±0·004917	±0·002899
Variance of mean .	0·00002818	0·00001042	0·00002417	0·00000840
Mean & S. E. difference . .	0·0069±0·0062		0·1044±0·0057	

Though the difference in grain length is not significant in the case of *Ngas in* the difference in mean length is highly significant in the case of *Theikpan Taungdeikpan*. The effect is in both cases for the grain lengths to decrease with wider spacing, while the number of grains and number of tillers per plant increases.

The effect on the degree of variablity is shown in Table XXII.

TABLE XXII

Ngasein *and* Theikpan Taungdeikpan, *differences in variability at 1 ft. × 1 ft. and 2 ft. × 2 ft. spacings*

Variety	Degrees of freedom	Sum of squares	Mean squares	$\frac{1}{2}$ $\log_e$ (mean square)	$\frac{1}{n}$
Ngasein 1 ft. × 1 ft. .	3462	337·97	0.09762	1·1392	0·0002889
Ngasein 2ft. × 2 ft. .	7991	635·92	0.07958	1·0371	0·0001251
Theikpan Taungdeikpan 1 ft. × 1 ft.	6681	681·22	0.10196	1·1610	0.0001497
Theikpan Taungdeikpan 2 ft. × 2 ft.	14451	1756·70	0.12156	1·2490	0·0000692

For *Ngasein* $\frac{1}{2}$ log difference is 0·1021 and S. D. of $z = 0·01438$

For *Taungdeikpan* $\frac{1}{2}$ $\log_e$ difference is 0·0880 and S. D. of $z = 0·01045$.

In both varieties the differences in variability are significant even within one variety, indicating that variability is easily affected by changes in spacing.

IX. Effect of harvesting date on grain size

There being no sharp indication of when paddy has reached a degree of ripeness most sutable for harvesting, there is in practice a great deal of uncertainty. It has been the practice in this laboratory to estimate life-period as the number of days from sowing to flowering *plus* thirty days, but this arbitrary period of thirty days for the grain filling and ripening period after flowering is in many cases too short. It holds for short-lived varieties of 150 days life-period or less, but is not correct for long-lived types. Further paddies of the same life-period often require different lengths of time from flowering to harvest. The exact stage at which paddy is reaped is held, with good reason, to affect its milling and quality, and the optimum stage is not the same for all. For instance, *Taungdeikpan* is usually reaped while distinctly unripe, whereas *Ngasein* is allowed to stand until dead ripe.

In 1931-32 *Ngasein 2104* was harvested at different dates after flowering and measurements of length and breadth were made. The figures are shown in Tables XXIII and XXIV.

Table XXIII

Ngasein 2104, *length of grain in mm. after harvesting on different dates*

Harvesting dates	32 days after flowering 11th Dec. 1931	36 days after flowering 15th Dec. 1931	40 days after flowering 19th Dec. 1931	44 days after flowering 23rd Dec. 1931	48 days after flowering 27th Dec. 1931
Standard deviation . .	0·28448	0·26418	0·28272	0·33564	0·31992
Mean	8·6004	8·6132	8·5588	8·5300	8·5368
S. E. of S. D. . . .	0·008996	0·008354	0·008941	0·010614	0·010116
S. E. of mean . . .	0·012722	0·011817	0·012643	0·015010	0·014307

TABLE XXIV

Ngasein 2104, *breadth of grain in mm. after harvesting on different dates*

Harvesting dates	11th Dec. 1931	15th Dec. 1931	19th Dec. 1931	23rd Dec. 1931	27th Dec. 1931
Standard deviation . .	0·11098	0·12590	0·11826	0·13169	0·12236
Mean	3·4532	3·4160	3·4010	3·3908	3·4132
S. E. of S. D. . . .	0·003509	0·003981	0·003740	0·004164	0·003869
S. E. of mean . . .	0·004961	0·005631	0·005289	0·005889	0·005472

TABLE XXV

Ngasein 2104, *mean differences and S. E. for length of grain when harvested on different dates*

Harvesting dates	15th Dec. 1931	19th Dec. 1931	23rd Dec. 1931	27th Dec. 1931
11th Dec. 1931	0·0128±0·017362	*0·0460±0·017936*	*0·0704±0·019677*	*0·0636±0·019145*
15th Dec. 1931		*0·0544±0·017305*	*0·0832±0·019100*	*0·0764±0·018553*
19th Dec. 1931			0·0288±0·019625	0·0220±0·019094
23rd Dec. 1931				0·0068±0·020736

Those printed in *italics* are significant.

TABLE XXVI

Ngasein 2104, *mean differences and S. E. for breadth of grain when harvested on different dates*

Harvesting dates	15th Dec. 1931	19th Dec. 1931	23rd Dec. 1931	27th Dec. 1931
11th Dec. 1931	*0·0372±0·007503*	*0·0522±0·007246*	*0·0624±0·007701*	*0·0400±0·00738*
15th Dec. 1931		0·0150±0.007720	*0·0252±0·008149*	0·0028±0·007849
19th Dec. 1931			0·0102±0·007912	0·0122±0·007603
23rd Dec. 1931				*0·0224±0·008037*

Those printed in *italics* are significant.

The measurements were done several months after harvest when the samples had reached the same stage of air-dryness. From the tables it will be seen that both dimensions have varied significantly in most cases. The tendency is for later harvesting to produce shorter and narrower grains.

When the effect of harvesting date on the degree of variability was measured, though some significant differences occurred, no general tendency could be discovered and it seems probable that the variations were due to chance.

X. EFFECT OF SEASON ON GRAIN WEIGHTS

The data were obtained from the Permanent Manurial Experiment on the Mandalay Agricultural College Farm. The details of the lay-out have been reported in the Annual Reports of the Agricultural College Farm, Mandalay, from 1927 onwards. Briefly there are seventy-four plots of 0·1 acre of which thirty-eight are untreated. Each treatment has been repeated four times and there is an un-treated plot between each pair of treated plots. The variety is *Ngasein 2104*. The treatments are :—

(*a*) Lime, 5 cwt. calcium oxide per acre every four years. Second application in 1930.

(*b*) Sodium nitrate, 40 lbs. nigtrogen per acre.

(*c*) Ammonium sulphate, 40 lbs. nitrogen per acre.

(*d*) Superphosphate, 40 lbs. phosphoric acid per acre.

(*e*) Bone-meal, 40 lbs. phosphoric acid per acre.

(*f*) Potassium sulphate, 40 lbs. potassium oxide per acre.

(*g*) Ammonium sulphate, 40 lbs. nitrogen per acre ; superphosphate, 40 lbs. phosphoric acid per acre.

(*h*) Ammonium sulphate, 40 lbs. nitrogen ; superphosphate, 40 lbs. phosphoric acid ; potassium sulphate, 40 lbs. potassium oxide per acre.

(*i*) Farmyard manure, 40 lbs. nitrogen per acre.

Manures are applied shortly before transplanting. The bone-meal used has been a very coarse grade hitherto.

Weights of hundred grains from each plot were taken in the seasons 1929-30, 1930-31 and 1931-32. All the thirty-eight controls were not treated separately but instead eighteen means were used, these being arrived at for each year by first taking the means of two adjacent controls lying on opposite sides of treated plots and then taking means of the two adjacent means of controls of the two rows, the experiment being laid out in two blocks of two replications each with an irrigation distributory running between.

TABLE XXVII

Permanent Manurial Series (weight of 100 grains of paddy of pooled means of controls)

Serial No.	1929-30	1930-31	1931-32	Total
1	2·947	2·865	2·945	8·757
2	2·994	2·928	2·925	8·847
3	2·921	2·953	2·936	8·810
4	2·924	2·920	2·959	8·803
5	2·965	2·930	2·956	8·851
6	2·943	2·903	2·962	8·808
7	2·911	2·883	2·977	8·771
8	2·918	2·933	2·980	8·831
9	2·943	2·910	2·978	8·831
10	2·948	2·900	2·960	8·808
11	2·928	2·937	2·928	8·793
12	2·948	2·923	2·990	8·861
13	2·890	2·870	2·990	8·750
14	2·932	2·840	2·960	8·732
15	2·916	2·868	2·974	8·758
16	2·857	2·863	2·987	8·707
17	2·878	2·880	3·008	8·766
18	2·911	2·907	3·016	8·834
Total .	52·674	52·213	53·431	158·318

TABLE XXVIII

Analysis of variance

Due to	Degrees of freedom	Sum of squares	Mean squares	$\frac{1}{2}$ $\log_e$ (mean square)	Remarks
Years	2	0·0421	0·021050	3·7390	Significant
Replications . . .	17	0·0110	0·000647	2·0850	
Residual . . .	34	0·0330	0·000970	2·1844	
Total .	53	0·0861	One per cent point is 0·8423		

The analysis of variance shows that seasonal effect on the weight is significant. The S. E. for the total of eighteen means being 0·132 the difference between any two years is significant. Of the three years, 1931-32 has given the heaviest grains and 1930-31 the lightest.

XI. EFFECT OF SEASON ON GRAIN LENGTH

Figures for the seasonal variation in grain length are available only for *Theikpan Taungdeikpan* for the years 1930, 1931 and 1933. The grains were taken from increase plots planted two ft. × one ft. and receiving the same treatments. The figures are shown in Tables XXIX and XXX.

TABLE XXIX

Theikpan Taungdeikpan, *seasonal variation in grain length (mm.)*

Year	1930-31	1931-32	1933-34
Mean	8·6008	8·7180	8·7530
S. E. of mean . . .	0·018090	±0·017039	±0·022175

TABLE XXX

Theikpan Taungdeikpan, *mean length differences and S. E. of differences*

Year	Means (mm.)	Difference from	
		1931-32	1933-34
1930-31 . . .	8·6008	*0·1172 ± 0·0227*	*0·1522 ± 0·0286*
1931-32 . . .	8·7180		0·0350 ± 0·0280

Those printed in *italics* are significant.

The difference between the means of 1933 and 1931 are not reliable, but in 1931 the length was definitely greater than in 1930, and also 1933 is significantly longer than in 1930. This agrees with the data for *Ngasein* grain weights.

Differences in degree of variability from year to year in grain lengths were not found to be significant and only in one case did variability in grain lengths show a reliable difference.

XII. EFFECT OF MANURE ON GRAIN WEIGHT

(a) *Paddy*

The weights of one hundred grains were taken from each plot of the Permanent Manurial Series. The means of each pair of controls on opposite sides of treated plots were used for comparisons. The figures relate to the years 1929-30, 1930-31 and 1931-32.

TABLE XXXI

Effect of manure on weight of paddy grains (grms.)

Treatment	Year	Mean weight (100 grains)		Percentage increase or decrease	Fisher's *t*
		Treated	Control		
Lime	1929-30	2·9655	2·9476	+0·6073	0·8476
	1930-31	2·8650	2·8825	—0·6071	0·1225
	1931-32	2·9823	2·9474	+1·1841	1·6530

TABLE XXXI—(*contd*)

Treatment	Year	Mean weight (100 grains)		Percentage increase or decrease	Fisher's *t*
		Treated	Control		
Sodium nitrate . . .	1929-30	2·9327	2·9610	—0·9558	0·9034
	1930-31	2·9125	2·9325	—0·6820	0·5123
	1931-32	2·9866	2·9484	+1·2956	1·9060
Ammonium sulphate . .	1929-30	2·9490	2·9341	+0·5078	0·4327
	1930-31	3·0075	2·9375	+2·3885	1·6847
	1931-32	3·0032	2·9632	+1·3499	2·1760
Superphosphate . .	1929-30	2·9402	2·9065	+1·1594	0·9908
	1930-31	2·9275	2·8950	+1·1226	1·2460
	1931-32	3·0022	2·9746	+0·9279	1·0034
Bone-meal . . .	1929-30	2·9527	2·9481	+0·1560	0·2046
	1930-31	2·9575	2·8850	+2·5130	1·8785
	1931-32	2·9811	2·9580	+0·7809	1·5860
Potassium sulphate . .	1929-30	2·9352	2·9297	+0·1877	0·2091
	1930-31	2·9025	2·8850	+0·4842	0·7285
	1931-32	2·9795	2·9677	+0·3968	0·3588
Ammonium sulphate + superphosphate	1929-30	2·9985	2·8842	+3·9630	*3·1640*
	1930-31	2·9600	2·8775	+2·8671	*5·1120*
	1931-32	3·0469	2·9869	+2·0087	2·2240
Ammonium sulphate + superphosphate + potassium sulphate	1929-30	3·0337	2·8975	+4·7006	*5·0160*
	1930-31	2·990	2·9062	+2·8835	2·1050
	1931-32	3·0486	2·9942	+1·8168	*3·2080*
Farmyard manure . .	1929-30	3·0162	2·9286	+3·0547	*2·6120*
	1930-31	2·9325	2·9087	+1·1621	0·6163
	1931-32	3·0376	2·9976	+1·3344	1·5770

Five per cent value of *t* is 2·4470.

In no case have dressings of single fertilizers given significant results. Ammonium sulphate in combination with superphosphate has given significant increases of mean grain weight in 1929-30 and 1930-31 but just fails to show a reliable increase in 1931-32, although the actual weight in the third year was the highest. In that year the mean weight of the adjacent controls was also unusually high. It is considered that there is little doubt that this combination of fertilizers causes a true increase in mean grain weight.

In the case of ammonium sulphate + superphosphate + potassium sulphate the increases are significant in 1929-30 and 1931-32. In 1930-31 inspite of an increase of 2·88 per cent the result is not reliable. There has been a decrease in the percentage increase in each suceeding year. This may be due to depletion of some nutrient other than those added in these fertilizers on account of the heavy yields of grain and straw from these plots.

Farmyard manure has given a significant increase only in the first year and it seems doubtful whether this manure has any definite effect on grain weight.

(*b*) *Effect of manures on weight of husked rice*

Husked rice (not polished) from the Permanent Manurial Series was compared in the same way as the paddy and the results are shown in Table XXXII.

TABLE XXXII

Effect of manure on weight of husked rice (Ngasein 2104)

Treatment	Year	Mean weight in grms. (100 grains)		Percentage increase or decrease	Fisher's *t*
		Treated	Control		
Lime	1929-30	2·2805	2·2774	+0·0136	0·1873
	1930-31	2·2750	2·2750	0	0
	1931-32	2·3683	2·3625	+0·2455	0·4544
Sodium nitrate . .	1929-30	2·2570	2·2724	—0·6777	1·1284
	1930-31	2·3050	2·3050	0	0
	1931-32	2·3549	2·3500	+0·2085	0·2896

TABLE XXXII-(*contd*)

Treatment	Year	Mean weight in gram (100 grains)		Percentage increase or decrease	Fisher's *t*
		Treated	Control		
Ammonium sulphate .	1929-30	2·2930	2·2691	+1·0533	1·6250
	1930-31	2·3600	2·3137	+2·0012	*3·2460*
	1931-32	2·3934	2·3502	+1·8381	2·0820
Superphosphate . .	1929-30	2·2962	2·2596	+1·6198	*2·9241*
	1930-31	2·3350	2·2937	+1·8006	1·9640
	1931-32	2·4045	2·3679	+1·5453	1·8630
Bone-meal . . .	1929-30	2·2732	2·2675	+0·2514	0·2940
	1930-31	2·3100	2·3087	+0·0563	0·5308
	1931-32	2·3610	2·3679	—0·2914	0·4526
Potassium sulphate . .	1929-30	2·2687	2·2655	+0·1413	0·1645
	1930-31	2·2850	2·2775	+0·3293	0·4789
	1931-32	2·3438	2·3538	—0·4248	0·9755
Ammonium sulphate+superphosphate	1929-30	2·3107	2·2537	+2·5292	*2·6960*
	1930-31	2·3450	2·2875	+2·5143	*2·7565*
	1931-32	2·4297	2·3778	+2·1827	*2·4705*
Ammonium sulphate+superphosphate+potassium sulphate	1929-30	2·3725	2·2622	+4·8759	*5·6313*
	1930-31	2·3627	2·3050	+2·7115	2·3635
	1931-32	2·4106	2·3870	+0·9886	1·6890
Farmyard manure . .	1929-30	2·3282	2·2789	+2·1634	1·5528
	1930-31	2·3150	2·3812	+1·2020	1·0963
	1931-32	2·4206	2·3828	+1·5864	*3·7490*

Five per cent value of *t* is 2·4470.

Lime, sodium nitrate, bone-meal and superphosphate appear to be definitely without effect. Although ammonium sulphate and superphosphate fail to show significant responses, the increases are of a size to suggest a real effect. Ammonium sulphate and superphosphate together give a definite increase in weight in every year but when potassium sulphate is added the increase is significant only in one year. Farmyard manure gives a significant increase in only one year.

(c) *Effect of manures on the weight of polished rice*

The weighings, etc., for polished rice from the Permanent Manurial Experiment are given in Table XXXIII.

TABLE XXXIII

The effect of manure on weight of polished rice (Ngasein 2104)

Treatments	Year	Mean weight in grms. (10 grains)		Percentage increase or decrease	Fisher's t
		Treated	Control		
Lime	1929-30	2·144	2·127	+0·0799	1·060
	1930-31	2·127	2·127	0	0
Sodium nitrate	1929-30	2·087	2·122	—1·6994	2·000
	1930-31	2·150	2·154	—0·1773	0·397
Ammonium sulphate	1929-30	2·164	2·122	+1·9792	*4·950*
	1930-31	2·210	2·156	+2·5046	*4·972*
Superphosphate	1929-30	2·140	2·109	+1·4698	1·750
	1930-31	2·170	2·129	+1·9258	2·345
Bone-meal	1929-30	2·156	2·112	+2·0834	1·373
	1930-31	2·155	2·144	+0·5131	0·352
Potassium sulphate	1929-30	2·122	2·116	+0·2835	0·3661
	1930-31	2·145	2·145	0	0
Ammonium sulphate + superphosphate	1929-30	2·164	2·116	+2·2684	*3·100*
	1930-31	2·207	2·144	+2·9385	*4·338*
Ammonium sulphate + superphosphate + potassium sulphate	1929-30	2·197	2·126	+3·3396	*2·636*
	1930-31	2·212	2·162	+2·3127	*2·811*
Farmyard manure	1929-30	2·175	2·133	+1·9690	1·430
	1930-31	2·150	2·137	+0·6083	0·552

Five per cent value of t is 2·4470

Lime, superphosphate, bone-meal and potassium sulphate increased the polished rice weights but the effect is not definite, while sodium nitrate decreased the weight though the effect was negligible. Ammonium sulphate alone and in combination produced definite effects on the polished rice weight. The effect of combined manures seemed to be additive. Farmyard manure failed to show a significant response in both years.

For comparison between one treatment and another and between years, the following modified method of calculation was done for analysis of variance.*

The weights of one hundred grains for three years each of seventy four plots were obtained. Means of the controls on either side of the treated were then worked out, so that nine means for controls for each replication of each treatment were obtained. Means of nine means were then calculated for four replications. Hence there remained four controls and thirty-six manured giving a total of forty figures for each year, *i.e.*, ten treatments of four replications. The weighings are tabulated in Table XXXIV.

* Though the " analysis of variance " method is not strictly applicable to the present case, an effort has been made in these tables to separate the several items by the above method.

TABLE XXXIV

Weight of paddy in grms.

Treatments	Lime	Sodium-nitrate	Am. sulphate	Super phosphate	Bone-meal	Potassium sulphate	Am. sulphate + super	Am. sulphate + super + potassium sulphate	F. Y. M.	Control	Total
1929-30	2·960	2·903	2·900	2·932	2·972	2·963	2·953	3·035	2·990	2·947	29·455
	2·942	2·908	2·958	2·873	2·917	2·975	2·982	3·000	2·950	2·870	29·375
	2·963	2·950	2·980	2·978	3·000	2·948	3·022	3·060	3·030	2·826	29·757
	2·997	2·970	2·958	2·978	2·922	2·955	3·037	3·040	3·095	2·949	29·901
Total	11·862	11·731	11·796	11·761	11·811	11·741	11·994	12·135	12·065	11·592	118·488
Mean	2·965	2·933	2·949	2·940	2·953	2·935	2·998	3·034	3·016	2·898	
1930-31	2·860	2·850	2·940	2 950	3·020	2·900	2·970	3·010	2·930	2·919	29·349
	2·950	2·910	2·970	2·900	2·900	2·850	2·980	2·950	2·840	2·867	29·117
	2·750	2·850	3·000	2·910	2·990	2·940	2·950	3·050	2·980	2·980	29·330
	2·900	3·040	3·120	2·950	2·920	2·920	2·940	2·950	2·990	2·908	29·638
Total	11·460	11·650	12·030	11·710	11·830	11·610	11·840	11·960	11·740	11·604	117·434
Mean	2·865	2·912	3·007	2·927	2·958	2·902	2·960	2·990	2·935	2·901	
1931-32	2·995	2·968	3·000	2·929	3·001	2·901	3·019	3·019	3·059	2·948	29·839
	3·021	2·978	2·994	3·004	2·978	3·002	2·998	3·045	3·020	2·967	30·007
	2·926	2·969	3·008	3·033	2·983	2·978	3·037	3·063	2·987	2·945	29·979
	2·987	3·031	3·011	3·042	2·963	3·037	3·084	3·067	3·084	3·003	30·309
Total	11·929	11·946	12·013	12·008	11·925	11·918	12·138	12·194	12·154	11·863	120·134
Mean	2·982	2·986	3·003	3·002	2·981	2·979	3·047	3·048	3·036	2·965	
Grand Total	35·251	35·327	35·839	35·479	35·566	35·269	36·022	36·289	35·955	35·059	356·056

Analysis of variance for the year 1929-30 is given in Table XXXV.

TABLE XXXV

Analysis of variance (1929-30)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Loge (mean square)
Replication	3	0·018496	0·006165	4·3520
Treatments	9	0·063245	0·007027	4·4288
Residual	27	0·038191	0·001414	3·6270
Total .	39	0·119932		

One per cent value of z is 0·7631
5 per cent value of z is 0·5427
S. E. of mean of treatments is 0·0188

Though it does not quite agree with the result obtained by the method of paired differences in comparing with the control, yet in most cases the results agree with the general expectation. Effective manures give significant differences over controls.

TABLE XXXVI

Final results for 1929-30 (weight of paddy)

Treatments	Mean weights	Difference from								
		1	2	3	4	5	6	7	8	9
Control	2·898									
Lime	2·965	*+0·067*								
Sodium nitrate	2·933	+0·035	—0·032							
Ammonium sulphate	2·949	*+0·051*	—0·016	+0·016						
Superphosphate	2·940	*+0·042*	—0·025	+0·007	—0·009					
Bone-meal	2·953	*+0·055*	—0·012	+0·020	+0·004	+0·013				
Potassium sulphate	2·935	+0·037	—0·030	+0·002	—0·014	—0·005	—0·018			
Am. sulph.+super	2·998	*+0·100*	+0·033	*+0·065*	*+0·049*	*+0·058*	*+0·045*	*+0·063*		
Am. sulph+super.+pot.	3·034	*+0·136*	*+0·069*	*+0·101*	*+0·088*	*+0·094*	*+0·081*	*+0·099*	+0·036	
Farmyard manure	3·016	*+0·118*	*+0·059*	*+0·083*	*+0·067*	*+0·076*	*+0·063*	*+0·081*	+0·018	—0·018

For five per cent point the difference must exceed 0·038577

It is clear from the above that increase in weight is considerable and significant in cases of effective manures such as ammonium sulphate and superphosphate. Potassium sulphate increases the weight but not significantly. The effect of combined manures appears to be roughly additive.

Increase due to ammonium sulphate alone . . .	=0·051
Increase due to superphosphate alone . . .	=0·042
Sum .	=0·093
Increase due to ammonium sulphate and superphosphate together	=0·100
Difference .	=0·007

The analysis of variance for the year 1930-31 is given in Table XXXVII.

TABLE XXXVII

Analysis of variance for 1930-31 (weight of paddy)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Loge (mean square)
Replications . . .	3	0·013734	0·004578	4·2146
Treatments	9	0·068320	0·007591	4·4674
Residual	27	0·130851	0·004846	4·2430
Total .	39	0·212905	'z-test fails	

S. E. of the mean of treatments = 0.034799.

TABLE XXXVIII

Final results for 1930-31 (weight of paddy)

Treatments	Mean weight	Difference from treatment								
		1	2	3	4	5	6	7	8	9
Control	2·901									
Lime	2·865	—0·036								
Sodium nitrate	2·912	+0·011	+0·047							
Ammonium sulphate	3·007	+0·106	*+0·142*	*+0·095*						
Superphosphate	2·927	+0·026	+0·062	+0·015	*—0·080*					
Bone-meal	2·958	+0·057	*+0·093*	+0·046	—0·049	+0·031				
Potassium sulphate	2·902	+0·001	+0·037	—0·010	*—0·105*	—0·025	—0·056			
Am. sulph.+super.	2·960	+0·059	*+0·095*	+0·048	—0·047	+0·033	+0·002	+0·058		
Am. sulph.+super.+pot.	2·990	*+0·089*	*+0·125*	*+0·078*	—0·017	+0·063	+0·032	*+0·085*	+0·030	
Farmyard manure	2·935	+0·034	+0.070	+0·023	*—0·072*	+0·008	—0·023	+0·033	—0·025	—0·055

For five per cent point the difference must exceed 0.07148

In the year 1930-31 ammonium sulphate alone gave the largest increase while lime had a negative effect though not significant.

The analysis of variance for the year 1931-32 is given in Table XXXIX.

TABLE XXXIX

Analysis of variance for 1931-32 (weight of paddy)

Due to	Degrees of freedom	Sum of squares	Mean square	½ Loge (mean square)
Replications . . .	3	0·010740	0·003580	4·0917
Treatments	9	0·033748	0·003749	4·1147
Residual	27	0·029507	0·001093	3·4982
Total .	39	0·073995	*z*-test passes point	5 per cent

S. E. of mean of treatments is 0·0165287

TABLE XL

Final result for 1931-32 (weight of paddy)

Treatments	Mean weights	Difference from treatment								
		1	2	3	4	5	6	7	8	9
Control	2·965									
Lime	2·982	+0·017								
Sodium nitrate	2·986	+0·021	+0·004							
Ammonium sulphate	3·003	*+0·038*	+0·021	+0·017						
Superphosphate	3·002	*+0·037*	+0·020	+0·016	—0·001					
Bone-meal	2·981	+0·016	—0·001	—0·005	—0·022	—0·021				
Potassium sulphate	2·979	+0·014	—0·003	—0·007	—0·024	—0·023	—0·002			
Amm. sulph.+super.	3·047	*+0·082*	*+0·065*	*+0·061*	*+0·044*	*+0·045*	*+0·066*	*+0·068*		
Amm. sulph.+super.+pot.	3·048	*+0·083*	*+0·066*	*+0·062*	*+0·045*	*+0·046*	*+0·067*	*+0·069*	+0·001	
Farmyard manure	3·037	*+0·072*	*+0·055*	*+0·051*	*+0·034*	*+0·035*	*+0·056*	*+0·058*	—0·010	—0·011

For five per cent point the difference must exceed 0·03395

The final results of 1931-32 agree closely with the results of 1929-30. Effective manures have a definite effect on the weights. Ammonium sulphate, superphosphate and potassium sulphate were additive in effect. Farmyard manure seems to have about the same effect as the combined effect of the three manures.

Figures for the three years were combined and statistical analysis was done by the method of analysis of variance into parts.

The analysis is given in Table XLI, and the final result for comparison is given in Table XLII.

TABLE XLI

Analysis of variance. Weight of paddy (three years combined)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Log$_e$ (mean square)	Value of z
Years. . . .	2	0·072593	0·036296	5·2496	1·7266
Replication. . 3					
Interaction between year and replication . 6	9	0·062971			
Treatment . . .	9	0·251675	0·279640	5·1192	1·5962
Residual . . .	99	0·113184	0·001143	3·5230	
Total .	119	0·500423			

One per cent value of z for years is 0·7636
One per cent value of z for treatments is 0·4604
S. E. of mean of four plots (treatments) is 0·01868
S. E. of total of twelve plots is 0·1290
S. E. of total of forty plots is 0·2363
S. E. of total of ten plots is 0·1180

Grain weight was highest in 1931-32 and lowest in 1930-31. The differences must be judged significant.

Final result for comparison between the treatments in different years and between treatment totals for three years is given in Table XLII.

TABLE XLII

Final results

Mean of four plots	Control	Lime	Sodium nitrate	Ammonium sulphate	Super-phosphate	Bone-meal	Pot. sulphate	Am. sulph. +super.	Am. sulph. +sulper +pot. sulph.	F. Y. M.	S. E.
1929-30	2·898	2·965	2·933	2·949	2·940	2·953	2·935	2·998	3·034	3·016	
1930-31	2·901	2·865	2·917	3·007	2·927	2·958	2·902	2·960	2·990	2·935	0·01868
1931-32	2·965	2·982	2·986	3·003	3·002	2·981	2·979	3·047	3·048	3·037	
Total of three years (total of 12 plots)	35·059	35·251	35·327	35·839	35·479	35·566	35·269	36·022	36·289	35·955	0·12900

Of the manures tested ammonium sulphate and its combinations gave the heaviest grain. Superphosphate and Farmyard manure, the latter containing the combination of the three effective manures, gave considerable increases. Lime and potassium sulphate on the whole were not effective, while sodium nitrate and bone-meal gave effective response to a considerable extent. Ammonium sulphate and its combinations show significant increases over other manures such as lime, sodium nitrate and potassium sulphate.

The weighings of husked rice are given in Table XLIII.

TABLE XLIII

Weights of husked rice

Treatments	Control	Lime	Sodium nitrate	Ammonium sulphate	Superphosphate	Bone-meal	Pot. sulph.	Am. sulph. +super	Am. sulph. +super +pot. sulph.	F. Y. M.	Total
1929-30	2·275	2·288	2·253	2·283	2·300	2·282	2·257	2·343	2·333	2·283	22·897
	2·242	2·238	2·240	2·277	2·305	2·248	2·230	2·275	2·363	2·300	22·718
	2·279	2·293	2·250	2·322	2·300	2·310	2·283	2·335	2·397	2·310	23·079
	2·272	2·303	2·285	2·290	2·280	2·253	2·305	2·290	2·397	2·420	23·095
Total .	9·068	9·122	9·028	9·172	9·185	9·093	9·075	9·243	9·490	9·313	91·789
Mean	2·267	2·280	2·257	2·293	2·296	2·273	2·269	2·311	2·372	2·328	
1930-31	2·299	2·280	2·260	2·340	2·330	2·330	2·280	2·350	2·360	2·290	23·119
	2·273	2·290	2·320	2·340	2·310	2·320	2·270	2·340	2·340	2·260	23·063
	2·299	2·220	2·300	2·370	2·310	2·330	2·300	2·380	2·380	2·370	23·259
	2·302	2·310	2·340	2·390	2·390	2·260	2·290	2·310	2·390	2·340	23·322
Total .	9·173	9·100	9·220	9·440	9·340	9·240	9·140	9·380	9·470	9·260	92·763
Mean	2·293	2·275	2·305	2·360	2·335	2·310	2·285	2·345	2·367	2·315	
1931-32	2·351	2·351	2·381	2·385	2·367	2·381	2·305	2·415	2·407	2·412	23·755
	2·377	2·383	2·303	2·410	2·383	2·337	2·361	2·390	2·394	2·403	23·741
	2·365	2·359	2·355	2·394	2·425	2·334	2·355	2·447	2·413	2·436	23·883
	2·376	2·381	2·381	2·335	2·443	2·392	2·356	2·467	2·429	2·432	24·042
Total .	9·469	9·474	9·420	9·574	9·618	9·444	9·377	9·719	9·643	9·683	95·421
Mean	2·367	2·368	2·355	2·393	2·404	2·361	2·344	2·430	2·411	2·421	
Grand total .	27·710	27·696	27·668	28·186	28·143	27·777	27·592	28·342	28·603	28·256	279·973

The analysis of variance is given in Table XLIV and final results for comparison in Table XLV.

TABLE XLIV

Analysis of variance (Weight of husked rice, 1929-30)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Log$_e$
Replications	3	0·009427	0·003142	
Treatments	9	0·018860	0·002095	3·8238
Residual	27	0·045085	0·001669	3·7100
Total .	39	0·073372	z-test fails	

S. E. of mean of treatments is 0·0204

TABLE XLV

Final result (1929-30) (weight of husked rice)

Treatments	Mean weights	Difference from treatment								
		1	2	3	4	5	6	7	8	9
1. Control	2·267									
2. Lime	2·280	+0·013								
3. Sodium nitrate	2·257	—0·010	—0·023							
4. Ammonium sulphate	2·293	+0·026	+0·013	+0·036						
5. Superphosphate	2·296	+0·029	+0·016	+0·039	+0·003					
6. Bone-meal	2·273	+0·006	—0·007	+0·016	—0·020	—0·023				
7. Potassium sulphate	2·269	+0·002	—0·011	+0·012	—0·024	—0·027	—0·004			
8. Amm. sulph. +super.	2·311	*+0·044*	+0·031	*+0·054*	+0·018	+0·015	+0·038	*+0·042*		
9. Amm. sulph. +super +pot.	2·372	*+0·105*	*+0·092*	*+0·115*	*+0·079*	*+0·076*	*+0·099*	*+0·103*	*+0·061*	
10. Farmyard manure	2·328	*+0·061*	*+0·048*	*+0·071*	+0·035	+0·032	*+0·055*	*+0·059*	+0·017	*—0·044*

For five per cent point the difference must exceed 0·0418608

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Over the control the eighth, ninth and tenth treatments gave significant increases. Ammonium sulphate, superphosphate and potassium sulphate did not produce a substantial effect separately, but in combination a considerable response was obtained. Farmyard manure gave the second largest increase on the weight of husked rice. Sodium nitrate alone decreased the weight of husked rice.

The analysis of variance for the year 1930-31 is given in Table XLVI, and the final result is given in Table XLVII.

TABLE XLVI

Analysis of variance, 1930-31 (weight of husked rice)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ $\log_e$
Replications . . .	3	0·004335	0·001445	
Treatment	9	0·036053	0·004006	4·1479
Residual	27	0·025493	0·000944	3·4250
Total .	39	0·065881	z-test passes one per cent point	

S. E. of mean of treatments is 0·0154

TABLE XLVII

Final result for 1930-31 (weight of husked rice)

Treatments	Mean weight	Difference from treatment								
		1	2	3	4	5	6	7	8	9
1. Control	2·293									
2. Lime.	2·275	—0·018								
3. Sodium nitrate	2·305	+0·012	+0·030							
4. Ammonium sulphate	2·360	*+0·067*	*+0·085*	*+0·055*						
5. Superphosphate	2·335	*+0·042*	*+0·060*	+0·030	—0·025					
6. Bone-meal	2·310	+0·017	*+0·035*	+0·005	*—0·050*	—0·025				
7. Potassium sulphate	2·295	—0·008	+0·010	—0·020	*—0·075*	*—0·050*	—0·025			
8. Amm. sulph. + super	2·345	*+0·052*	*+0·070*	*+0·040*	—0·015	+0·010	*+0·035*	*+0·060*		
9. Amm. sulph. + super + pot. sulph	2·367	*+0·074*	*+0·092*	*+0·062*	+0·007	*+0·032*	*+0·057*	*+0·082*	+0·022	
10. Farmyard manure	2·315	+0·022	*+0·040*	+0·010	*—0·045*	—0·020	+0·005	+0·030	—0·030	*—0·052*

For five per cent point the difference must exceed 0·0316008

The value of z being significant, the result given above is reliable, while the result given in Table XLV, though taken into consideration, is not reliable.

From these results it is seen that, lime and potassium sulphate reduced the mean weight but not significantly, while ammonium sulphate, superphosphate and their combinations show a definite increase.

Analysis for the year 1931-32 is given in Table XLVIII, and the final result in Table XLIX.

TABLE XLVIII

Analysis of variance, 1931-32 (weight of husked rice)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ $\log_e$
Replications . . .	3	0·005875	0·001985	
Treatments	9	0·032469	0·003607	4·0953
Residual . . .	27	0·055273	0·002047	3·8121
Total .	39	0·093617	z-test fails	

S. E. of mean of treatment is 0·0226

TABLE XLIX

Final results for 1931-32 (weight of husked rice)

Treatment	Mean weight	Difference from treatment								
		1	2	3	4	5	6	7	8	9
1. Control	2·367									
2. Lime	2·368	+0·001								
3. Sodium nitrate	2·355	—0·012	—0·013							
4. Ammonium sulphate . . .	2·393	+0·026	+0·025	+0·038						
5. Superphosphate	2·404	+0·037	+0·036	*+0·049*	+0·011					
6. Bone-meal	2·361	—0·006	—0·007	+0·006	—0·032	—0·043				
7. Potassjum Sulphate	2·344	—0·023	—0·024	—0·011	*—0·049*	*—0·060*	—0·017			
8. Ammonnium sulphate +sper . .	2·430	*+0·063*	*+0·062*	*+0·075*	+0·037	+0·026	*+0·069*	*+0·086*		
9. Ammonium sulphate +super. +pot. sulph.	2·411	+0·044	+0·043	*+0·056*	+0·018	+0·007	*+0·050*	*+0·067*	—0·019	
10. Farmyard manure	2·421	*+0·054*	*+0·053*	*+0·066*	+0·028	+0·017	*+0·060*	*+0·077*	—0·009	+0·010

For five per cent point the differences must exceed 0·0463752.

Effective manures gave definite increases in grain weight, while the effect of other manures were not significant. In both paddy and husked rice, comparison shows that the result of 1929-30 agrees with the result of 1931-32 though in both cases the z-tests failed.

Analysis for the three years combined is given in Table L.

TABLE L

Analysis of variance (weight of husked rice) (Three years combined)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Log$_e$	z
Years . . .	2	0·176709	0·088354	3·3150	2·3082
Replications . 3					
Interaction between years and replication . 6	9	0·019637			
Treatments . .	9	0·139093	0·015454	2·5202	1·5134
Residual . . .	99	0·074140	0·000749	1·0068	
Total .	119	0·409579			

Five per cent value of z = 0·5486 (for years)
One per cent value of z = 0·7636
Standard error of mean of four plots = 0·01513
Standard error of total of twelve plots = 0·10478
Standard error of total of ten plots = 0·095655
Standard error of total of forty plots = 0·191311

The final result for comparison between years is given in Table LI.

TABLE LI

Year	1929-30	1930-31	1931-32	S. E.
Total of 40 means . .	91·789	92·763	95·421	0·191311

There has been an increase in total husked rice weight from 1929-30 onwards and all difference must be judged significant. This does not coincide with the results for paddy weight.

The final result for comparison between treatments of different years and between treatment totals for three years is given in Table LII.

TABLE LII

Combined final result for three years (husked rice weight)

Mean of four plots	Control	Lime	Sodium nitrate	Ammonium sulphate	Superphosphate	Bone-meal	Pot. sulphate	Am. sulph. +super.	Am. sulph. +super +pot. sulph.	F. Y. M.	S. E.
1929-30	2·267	2·280	2·257	2·293	2·296	2·273	2·269	2·311	2·372	2·328	
1930-31	2·293	2·275	2·305	2·360	2·335	2·310	2·285	2·345	2·367	2·315	0·01513
1931-32	2·367	2·368	2·355	2·393	2·404	2·361	2·344	2·430	2·411	2·421	
Total for three years . .	27·710	27·696	27·668	28·186	28·143	27·777	27·592	28·342	28·603	28·256	0·10478

The difference between the same treatment in different years is in most cases significant, especially between the first year and the third year of the experiment.

Comparing the totals ammonium sulphate, superphosphate, their combinations and farmyard manure gave effective responses, while the others did not.

XIII. Effect of manure on the size of grain

The data for calculation were obtained from the Permanent Manurial Experiment. The measurements are the means of 100 grains.

The results (for one year only, 1931-32) are shown in Tables LV, LVI, LVII and LVIII.

Table LV

Length of paddy grains in mm, Ngasein, 2104

Treatment	Mean length of paddy grain in mm.	Percentage increase or decrease	Fisher's *t*
Control	8·3448	+0·03955	0·12378
Lime	8·3481		
Control	8·3210	+0·31370	1·26840
Sodium nitrate	8·3471		
Control	8·3155	+0·80570	2·34530
Ammonium sulphate	8·3825		
Control	8·3181	+0·61790	1·52700
Superphosphate	8·3695		
Control	8·3240	+0·42170	1·51340
Bone-meal	8·3591		
Control	8·3373	+0·25070	0·68900
Potassium sulphate	8·3582		
Control	8·3293	+0·64230	*3·04850*
Ammonium sulphate + superphosphate.	8·3828		
Control	8·3262	+0·74220	*3·58200*
Ammonium sulph.+super+pot.	8·3880		
Control	8·3306	+0·67580	1·78900
Farmyard manure	8·3869		

Five per cent value of $t = 2·4470$

All the manures have increased the length of paddy, but only two treatments give significant results. Ammonium sulphate alone seemed to have a large effect though statistically not definite, but the addition of other slightly effective manures have increased the length definitely.

TABLE LVI

Breadth of paddy grains in mm. (Ngasein 2104)

Treatments	Mean breadth of paddy in mm.	Percentage increase or decrease	Fisher's *t*
Control	3·3638	+0·27945	0·5562
Lime	3·3732		
Control	3·3666	+0·11880	0·42215
Sodium nitrate	3·3706		
Control	3·3770	+0·29908	1·93020
Ammonium sulphate	3·3871		
Control	3·3790	+0·23380	1·22800
Superphosphate	3·3869		
Control	3·3746	—0·01778	0·10223
Bone-meal	3·3740		
Control	3·3805	—0·08579	0·15960
Potassium sulphate	3·3776		
Control	3·3843	+0·39594	1·80480
Ammonium sulphate + superphosphate.	3·3977		
Control	3·3803	+0·75920	*2·75340*
Ammonium sulphate + super + pot. sulphate.	3·4060		
Control	3·3747	+0·71120	*2·47740*
Farmyard manure	3·3987		

Five per cent value of $t = 2·4470$

Only two treatments definitely affected the breadth of paddy. Potassium sulphate and bone-meal reduced the breadth, but not significantly. The combination of three manures had a definite effect on both the length and breadth of paddy grains.

TABLE LVII

Length of husked rice in mm. (Ngasein)

Treatments	Mean length of husked rice in mm.	Percentage increase or decrease	Fisher's *t*
Control	5·9182	+0·3565	0·9069
Lime	5·9373		
Control	5·9183	+0·3481	1·9294
Sodium nitrate	5·9389		
Control	5·9142	+1·3425	*2·5461*
Ammonium sulphate	5·9936		
Control	5·9227	—0·2972	0·5709
Superphosphate	5·9051		
Control	5·9333	—0·0792	0·2480
Bone-meal	5·9286		
Control	5·9194	—0·3581	1·3944
Potassium sulphate	5·8982		
Control	5·9145	+0·9975	*3·8012*
Ammonium sulphate + superphosphate.	5·9735		
Control	5·9213	+0·5421	0·9680
Ammonium sulphate + super + pot. sulphate.	5·9534		
Control	5·9302	+0·4924	1·3235
Farmyard manure	5·9594		

Five per cent value of t = 2·4470

Superphosphate, bone-meal and potassium suplphate reduced the length, though the decreases are not significant. Ammonium sulphate alone showed the highest percentage increase over the control. By the addition of superphosphate, the increase in length was reduced. The increase in length was further reduced by the addition of potassium sulphate. The additive effect of the three manures was definite.

TABLE LVIII

Breadth of husked rice in mm. (Ngasein 2104)

Treatments	Mean breadth in mm.	Percentage increase or decrease	Fisher's *t*
Control	2·8441		
		+0·41840	0·7012
Lime	2·8560		
Control	2·8507		
		+0·16840	0·2617
Sodium nitrate	2·8555		
Control	2·8513		
		+0·9259	1·6163
Ammonium sulphate	2·8777		
Control	2·8498		
		+0·5931	1·1327
Superphosphate	2·8667		
Control	2·8493		
		−0·2492	0·6995
Bone-meal	2·8422		
Control	2·8484		
		+0·3862	0·6735
Potassium sulphate	2·8594		
Control	2·8608		
		+0·9648	1·7742
Ammonium sulphate + superphosphate.	2·8884		
Control	2·8683		
		+0·7809	2·0306
Ammonium sulphate + super + pot. sulphate.	2·8907		
Control	2·8631		
		+0·6986	1·7804
Farmyard manure	2·8831		

Five per cent value of t = 2·4470.

None of the treatments was definitely effective on the breadth of husked rice. Ammonium sulphate and its combinations increased the breadth, but the increases cannot be judged significant. On the whole there is distinct evidence that those manures which are effective in increasing grain yields also cause increases in the mean weights of grains. It has also been shown by Rhind [1930, 1932] that these manures tend to increase the liability to breakage of grain when the milling is carried out in a treadle pounder (Burmese *Moung*).

XIV. Effect of manure on the different parts of the paddy grain

Information as to how manures affect the different parts of the paddy grain was obtained by subtracting from the paddy weight, the husked and polished rice weights. The milling was done according to the method described by Rhind and U Tin [1933] with the apparatus described by Hayes [1932]. The results for 1929-30 and 1930-31 are shown in Tables LIII and LIV. Husk is estimated by difference of paddy and husked rice weights and bran by difference between husked and polished rice weights.

TABLE LIII

Effect of manures on weights of husk, bran, and polished rice (Ngasein) *1929-30*

Treatments	Husk			Bran			Polished rice		
	Control	Treated	Fisher's *t*	Control	Treated	Fisher's *t*	Control	Treated	Fisher's *t*
1. Lime	0·670 Increase	0.685 (+0·015)	1·2060 (N)	0·148 Decrease	0·137 (—0·011)	0·8215 (N)	2·127 Increase	2·144 (+0·017)	1·0600 (N)
2. Sodium nitrate	0·688 Decrease	0·675 (—0·013)	0·5167 (N)	0·150 Increase	0·170 (+0·020)	0·8468 (N)	2·122 Decrease	2·087 (—0·035)	2·0000 (N)
3. Ammonium sulphate	0·665 Decrease	0·656 (—0·009)	0·3353 (N)	0·148 Decrease	0·129 (—0·019)	1·3600 (N)	2·122 Increase	2·164 (+0·042)	4·9500 (S)
4. Superphosphate	0·647 Decrease	0·646 (—0·001)	0·0319 (N)	0·151 Increase	0·156 (+0·005)	0·3707 (N)	2·109 Increase	2·140 (+0·031)	1·7500 (N)
5. Bone-meal	0·681 Decrease	0·680 (—0·001)	0·1280 (N)	0·155 Decrease	0·117 (—0·038)	1·7640 (N)	2·112 Increase	2·156 (+0·044)	1·3730 (N)
6. Potassium sulphate	0·664 Increase	0·666 (+0·002)	0·0559 (N)	0·147	0·147 Neither increase nor decrease	0	2·116 Increase	2·122 (+0·006)	0·3661 (N)
7. Ammonium sulphate + superphosphate	0·631 Increase	0·688 (+0·057)	1·6560 (N)	0·138 Increase	0·147 (+0·009)	0·5738 (N)	2·116 Increase	2·164 (+0·048)	3·1000 (S)
8. Ammonium sulphate + superphosphate + pot. sulphate.	0·635 Increase	0·664 (+0·029)	1·2300 (N)	0·136 Increase	0·173 (+0·037)	2·5070 (S)	2·126 Increase	2·197 (+0·071)	2·6360 (S)
9. Farmyard manure	0·650 Increase	0·688 (+0·038)	1·3900 (N)	0·145 Increase	0·153 (+0·008)	0·6210 (N)	2·133 Increase	2·175 (+0·042)	1·4300 (N)

(N) and (S) in the table mean 'not significant' and 'significant'.
Five per cent value of $t = 2·4470$.

TABLE LIV

Effect of manures on weights of husk, bran and polished rice (Ngasein) *1930-31*

Treatments	Husk			Bran			Polished rice		
	Control	Treated	Fisher's *t*	Control	Treated	Fisher's *t*	Control	Treated	Fisher's *t*
1. Lime	0·605 Decrease	0·587 (—0·018)	0·4452 (N)	0·147	0·147	...	2·127	2·127	...
				Neither decrease nor increase			Neither decrease nor increase		
2. Sodium nitrate	0·627 Decrease	0·607 (—0·020)	0·5810 (N)	0·151 Increase	0·155 (+0·004)	0·2389 (N)	2·154 Decrease	2·150 (—0·004)	0·3972 (N)
3. Ammonium sulphate	0·624 Increase	0·647 (+0·023)	0·6781 (N)	0·157 Decrease	0·150 —(0·007)	0·5885 (N)	2·156 Increase	2·210 (+0·054)	4·9720 (S)
4. Superphosphate	0·604 Decrease	0·592 (—0·012)	0·5610 (N)	0·165	0·165	...	2·129 Increase	2·170 (+0·041)	2·3450 (N)
				Neither decrease nor increase					
5. Bone-meal	0·576 Increase	0·647 (+0·071)	2·1270 (N)	0·165 Decrease	0·155 (—0·010)	0·4900 (N)	2·144 Increase	2·155 (+0·011)	0·3520 (N)
6. Potassium sulphate	0·607 Increase	0·617 (+0·025)	0·7404 (N)	0·132 Decrease	0·140 (+0·008)	0·4484 (N)	2·145	2·145	...
							Neither decrease nor increase		
7. Ammonium sulphate + super.	0·590 Increase	0·615 (+0·025)	1·5400 (N)	0·144 Decrease	0·137 (—0·007)	0·5362 (N)	2·144 Increase	2·207 (+0·063)	4·3380 (S)
8. Ammonium sulphate + super- + potassium sulphate.	0·601 Increase	0·622 (+0·021)	0·8210 (N)	0·142 Increase	0·155 (+0·013)	0·8056 (N)	2·162 Increase	2·212 (+0·050)	2·8110 (S)
9. Farmyard manure	0·632 Decrease	0·617 (—0·015)	0·7247 (N)	0·144 Increase	0·165 (+0·021)	1·0480 (N)	2·137 Increase	2·150 (+0·013)	0·5523 (N)

(N) and (S) in the table mean 'not significant' and 'significant'.
Five per cent value of $t = 2·4470$.

In neither year has any treatment shown a significant effect on the weight of husk nor on the weight of bran except the treatment No. 9 (a combination of three manures) in the year 1930-31. Ammonium sulphate and its combinations have a definite effect on the weight of polished rice in both years. Therefore the increase in weight of paddy and husked rice by the addition of manure was due more to the increase in weight of rice than to the increase in the other parts. The addition has thus been to the most useful part of the fruit.

XV. Differences in grain size due to locality

It has been found that within the same administrative circle one variety of paddy from different localities differs in length and breadth to a considerable extent. The author is indebted to U Shwe Yin, Dip. Agri., Senior Agricultural Assistant, Myaungmya, for the following data for the variety *Emata A 26-3*, a translucent-grained type. The paddy was grown at eleven centres within the Irrawaddy Circle (Delta). The figures are given in Table LIX.

TABLE LIX

Mean length of grain, standard error and mean variance in mm. (Emata A 26-3)

Localities	Tagundaing	Letthama	Central Farm, Myaungmya	Pandabut	Sangin	Pyapon	Mayan	Bassein	Yegyi	Wakema	Myaungmya
Mean . . .	7·349	7·310	7·409	7·280	7·379	7·460	7·286	7·333	7·338	7·365	7·424
S. E. of mean .	0·02605	0.03263	0·02727	0·02694	0·03141	0·02996	0·03111	0·03033	0·03408	0·02949	0·03024
Variance of mean .	0·0006787	0·0010647	0·0007439	0·0007257	0·0009863	0·0008977	0·0009677	0·0009198	0·0011610	0·0008704	0·0009149

Standard error of the differences of the means = 0.03141

To test the homogeneity of the whole set of 1100 measurements they are analysed below in Table LX.

TABLE LX

Analysis of variance, grain lengths of Emata A 26-3

	Degrees of freedom	Sum of squares	Mean square	S. D.	$\log_e$ S. D.
Within localities .	1089	99·3077	0·09119	0·30197	3.6514
Between localities	10	0·030239	0·00302	0·05499	1·7046
Total . .	1099	99·3379		Difference	$=1·9468=z$

Standard deviation of z is 0·2246

The difference observed is significant, therefore the whole set of values appears to be non-homogeneous with a variance of about 0·090388. The standard error of means=0·03019.

Variation in breadth of grains from A 26-3 *grown at eleven different localities.*

The mean and standard error of the mean are given in Table LXI.

TABLE LXI

Mean breadth of grain, standard error and mean variance in mm. (Emata A 26-3)

Localities	Tagundaing	Letthama	Central Farm, Myaungmya	Pandabut	Sangin	Pyapon	Mayan	Bassein	Yegyi.	Wakema.	Myaungmya
Mean . . .	2·592	2·554	2·546	2·598	2·619	2·606	2·578	2·635	2·597	2·620	2·589
S. E. of mean .	0·01004	0·00863	0.00851	0·00914	0·00930	0.00978	0·00994	0·00861	0·00892	0·00978	0·01036
Variance of mean .	0·0001010	0·0000745	0·0000725	0·0000836	0·0000871	0·0000957	0·0000988	0·0000742	0·0000796	0·0000957	0·0001075

Standard error of the differences of the means = 0·00985

Analysis of variance to test the homogeneity is given in Table LXII.

TABLE LXII

Analysis of variance, grain breadths of Emata A 26-3

Due to	Degrees of freedom	Sum of squares	Mean square	S. D.	$\log_e$ (S. D.)
Within localities .	1089	9·702000	0·008909	0·09438	2·2447
Between localities .	10	0·007340	0·000734	0·02709	0·9966
Total . .	1099	9·709340		Difference	=1·2481

Standard deviation of z = 0·2246

Like length of grains, the majority of the mean differences in breadth are significant, and the whole set of values seems to be non-homogeneous with a variance of about 0·008833. The S. E. of means = 0·009438.

Difference in variability due to localities

Analyses for grain length and breadth are given in Tables LXIII and LXVIV.

TABLE LXIII

Analysis of variance of variabilities for length (A 26-3)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ $\log_e$ (mean square)	$\frac{1}{n}$
Tagundaing . . .	99	6·7886	0·06855	0·96246	0·010101
Letthama . . .	99	10·6400	0·10747	1·18730	0·010101
Central Farm, Myaungmya	99	7·4380	0·07513	1·00870	0·010101
Pandabut . . .	99	7·2556	0·07330	0·99600	0·010101
Sangin	99	9·8626	0·09962	1·14930	0·010101
Pyapon	99	8·9766	0·09067	1·10230	0·010101
Mayan	99	9·6771	0·09775	1·14000	0·010101
Bassein	99	9·1977	0·09291	1·11450	0·010101
Yegyi	99	11·6122	0·11729	1·23120	0·010101
Wakema	99	8·7042	0·08792	1·08700	0·010101
Myaungmya . . .	99	9·1491	0·09242	1·11190	0·010101

Standard deviation of z = 0·1004

TABLE LXIV

Analysis of variance of variabilities for breadth (A 26-3)

Due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Log$_e$ (mean square)	$\frac{1}{n}$
Tagundaing . . .	99	1·010	0·010202	1·1613	0·010101
Letthama . . .	99	0·745	0·007525	1·0088	0·010101
Central Farm, Myaungmya	99	0·725	0·007323	0·9955	0·010101
Pandabut . . .	99	0·836	0·008444	1·0670	0·010101
Sangin	99	0·871	0·008798	1·0872	0·010101
Pyapon	99	0·957	0·009666	1·1343	0·010101
Mayan	99	0·988	0·009980	1·1452	0·010101
Bassein	99	0·742	0·007495	1·0070	0·010101
Yegyi	99	0·796	0·008040	1·0422	0·010101
Wakema	99	0·957	0·009666	1·1343	0·010101
Myaungmya . . .	99	1·075	0·010859	1·1925	0·010101

Standard deviation of z = 0·1004

Differences in variability in length are significant in a few cases, while in breadth the difference is definite only between the paddies grown at the Central Farm, Myaungmya, and Myaungmya. Therefore the variabilities in length and breadth due to localities cannot be judged significant on these data.

XVI. DISCUSSION

The results presented in this paper reveal several interesting features on the developmental variation in the paddy grain in relation to various environmental factors. This variation in grain dimensions, though not large, is of such a magnitude as to be of importance in milling.

It has been shown that in order to get a representative sample, it is necessary to take the measurements of at least 100 grains. Lord [1929] found that in order to get a representative sample, measurement could be restricted to twenty grains only. Ramiah [1933] concluded from his experiment, that the measurement of

six grains from the top and middle portions of the panicle, was sufficient. The present experiment, however, definitely shows that little reliance can be placed on a random sample which consists of fewer than 100 grains.

As regards the variation within a panicle, Ramiah [1933] mentions only the presence of a higher proportion of ill-filled and half-set grains in the lower portion of the panicle, while van der Stok [1912] found that the mean weight decreases downward on the panicle. The latter's finding has been confirmed in this paper. Size, which generally depends upon weight, shows a tendency to decrease downwards, but comparison between mean lengths of grains of the top and middle portions did not show a singificant difference.

The conclusion arrived at by Ramiah and Parthasarathi [1933] that grains in different tillers (panicles) of the same plant do not vary significantly in size, is not confirmed. In the present experiments on both the two pure-line paddies significant differences have been obtained not only between one panicle and another of the same plant in the majority of comparisons, but also between one plant and another of the same paddy. Therefore variations within a plant and within a variety are definite even in stabilized types which have been grown in pure culture for a number of years in the same place under identical conditions.

The effect of spacing on the size of grain is not definite, but there are indications showing that grains tend to be longer with closer spacing. Of the two experiments carried out here, in one, size of grain has shown a definite response to spacing.

On the effect of various manures tested, ammoniacal fertilizers alone and in combination with other artificials cause a true increase in mean whole grain weight. Significant increase of the weight of grain without husk is caused only by the addition of two fertilizers together, *viz.*, ammonium sulphate and superphosphate. Regarding the effect on the polished rice, the most important part of the fruit, ammonium sulphate and its combinations produce significant increases. Husk and bran are only affected slightly by the addition of fertilizers. The influence of nitrogenous fertilizers on wheat have been studied by Engledow and Ramiah [1930] and it has been found that grain size is much affected. Results obtained here agree with their findings. Paddy grain dimensions are positively affected by the addition of ammonium sulphate in combination with superphosphate and potassium sulphate.

In the study of the influence of different harvesting dates, the results obtained here show that grains tend to become smaller with later harvesting, but detailed investigation is necessary to properly assess the role of ripening on the modification of grain size.

Influence of soil conditions and season on grain size has not received the attention of many investigators working on paddy. Ramiah and Parthasarathi [1933] in their paper pointed out that grain size is liable to be affected by different soil conditions and seasons. In this paper, results are given to show that soil and season have definite effects on the size of grain.

The effect of various environmental causes on the variability of paddy grain length, which is of some importance for plant breeders, has never been studied before. Variability, the amount of variance, is much affected by small alterations of the environment. The variability of one strain may or may not be the same as that of another. The variability of two different paddies may not differ significantly, but the variability of the same strain may be altered considerably by changes in spacing, harvesting date, season and soil.

This work has been carried out under the supervision of Mr. D. Rhind, Economic Botanist, Burma, to whom the author is greatly indebted for his suggestions and assistance in writing this paper.

XVII. Summary

1. Figures are given to show that samples of less than 100 grains cannot be relied on to give a representative grain measurement.

2. It is shown that within a panicle grain length and weight decreases from the top downwards and that the lower part of a panicle bears more unfilled grains than the upper.

3. Grain length may differ significantly between panicles of the same plant.

4. The extent to which pure lines may vary is itself a variable character.

5. In the variety *Taungdeikpan* the wider the spacing the shorter the grains tend to be. *Ngasein* did not show this effect clearly.

6. *Ngasein* has shown a decrease in variability at two ft. × two ft. spacing as compared to one ft. × one ft. while *Taungdeikpan* showed an increase at the wider spacing.

7. Length and breadth of *Ngasein* grains were reduced by later harvesting dates.

8. Grain weight of *Ngasein* was found to be liable to significant variation from year to year and grain length of *Taungdeikpan* showed a similar effect.

9. In *Ngasein* fertilizers have affected grain weight and dimensions. In general those fertilizers which give substantial increases in yield have given significant increases in grain size. In the case of combined manures the effect seems to be additive.

10. The increase in grain weight caused by fertilizers is shown to be due mainly to increase in the endosperm (rice) and not to the husk or bran.

11. Grain length and breadth of the pure line *A26-3* from eleven localities in Lower Burma are shown to differ significantly from place to place. The amount of variability does not differ significantly from place to place.

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STATISTICAL NOTES FOR AGRICULTURAL WORKERS

No. 17.—ON THE INFLUENCE OF SHAPE AND SIZE OF PLOTS ON THE EFFECTIVE PRECISION OF FIELD EXPERIMENTS WITH *JUAR* (*ANDROPOGON SORGHUM*)

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INTRODUCTION

The efficiency of a field experiment depends on the standard error of the different estimates involved in the test, and it is therefore necessary that the experimental arrangement should be designed in such a way as to ensure the highest possible precision.

Among the many factors that contribute to the magnitude of the errors in agricultural field experiments the following three are of considerable importance :—

(1) Shape and size of the individual plot.
(2) Shape and size of the Block Division (for elimination of soil heterogeneity).
(3) The position and orientation of the plots and of the blocks in the experimental field.

A certain amount of work on the influence of the shape and size of individual plots on the magnitude of errors has been done both in India and abroad. The crops chosen were generally the cereals, potatoes or sugar-beets,

In the United States, Stephens and Vinall [1928] working at Chillicothe-Texes in 1928 found three or four replications of 1/40 acre and 1/80 acre plots fully adequate for field tests with sorghum. Swanson [1930] has shown that the standard error of an experiment does not appreciably decrease after the plot size has increased to 1/25 acre ; he also observed that longer plots gave higher precision than square plots of equal area. Ligon [1930] has calculated with data on cotton grown on the Oklahoma Experimental Station that for optimum accuracies, the length of a plot did not exceed 100 feet.

In India, a uniformity trial with sugarcane (1933) was analysed and the results showed 1/24 acre (151'·3 × 12') to be the optimum area of plots for this crop. A more extensive experiment on rice at Chinsurah [Mahalanobis *et al*, 1934] (Bengal) Government Farm (2) showed the optimum area for rice to be 1/60 acre. In Assam [Mitra and Ganguli, 1934] again, the optimum area for rice was very nearly the same.

It is obvious that the optimum size is determined by the distribution of fertility contours in the given piece of land, and hence is likely to vary from place to place. But if the experimental site is kept fixed, the results of uniformity trials in any one year may be a valuable guide for work in the succeeding year, provided of course that the fertility distribution remains more or less the same from year to year. In the present investigation an attempt has been made to study these questions with the help of the results of an experiment with *Juar* conducted in Sholapur district for three consecutive years (1930-33).

Experimental details

One acre of land (290'.4 × 150') was chosen in the midst of a bigger area (plot No. 13 on the Mohol Plot) for sowing *Maldandi juar*, a variety extensively grown all over the Sholapur district. It was harvested in plots of 1/160 acre (72 ft. 6 in. × 3 ft. 9 in.) each containing three rows of the plants 15 in. apart. The 160 plots were arranged in forty rows of four columns, and the yields were measured in tolas. The plot division was kept intact for three years, and the yields of the 160 plots are available for three consecutive harvests. The original data are given in Appendix I.

Nature of distribution of experimental results

It has been claimed by the statistical workers at Rothamsted on the evidence of the experimental results of uniformity trials that the usual yield data obey approximately the Normal law of distribution. The data for rice yields at Chinsurah Farm have also broadly confirmed this result ; the deviations from the Normal distribution although significant are not large. In view of the importance of the question, the distribution of the *juar* yields has been investigated in detail. The frequency constants are given in Table I.

TABLE I

Frequency constants of the distribution of juar *with their standard errors*

	1930-31	1931-32	1932-33
n	160	160	160
Mean	116·2875±2·3851	67·2250±1·8765	126·3688±2·5501
Standard deviation	30·1695±1·6865	23·7365±1·3269	32·2568±1·8032
β_1	0·7930±0·2937	0·4339±0·4005	0·0802±0·2123
β_2	3·3107±0·5355	3·9002±1·5594	4·0205±2·1994

The distributions seem to be of the non-normal type ; but the values of β_1 and β_2 being based on only 160 observations have large standard errors. It is thus not possible to assert with these sets of observations that the distributions of the yields are significantly different from the normal type. Only in the first year, is the value of β_1 appreciably different from 0, *i.e.*, the skewness is significant In the two later years however it is fairly symmetrical. The values of β_2 although numerically greater than three in all the cases, cannot be considered to be different from three in the light of the standard errors. The observed distributions therefore may be taken to be a sample from a normal population. It may however be added that Egon Pearson* [1929] and others have shown on the basis of experimental sampling that the usual tests of significance are not invalidated for slight divergences from normal distribution.

ANALYSIS OF RESULTS

In analysing the data, fifteen different shapes of plot have been considered. It will be observed from the data given in Appendix I that heterogeneity is more marked between columns than between rows of same area. Thus in 1930-31, the estimated variance of the means of four columns is 512.6, while that of the means of four blocks (of ten rows each) is 102·5. The columns, singly and sometimes

* Egon S. Pearson assisted by Adyanthaya and others studied by actual sampling experiments the frequency distributions of Student's *z*, the usual test of significance between two observed means drawn from a number of non-normal symmetrical and skew populations given by (1) $\beta_1 = 0$, $\beta_2 = 2·5$, (2) $\beta_1 = 0$, $\beta_2 = 4·12$, (3) $\beta_1 = 0$, $\beta_2 = 7·07$ (4) $\beta_1 = 0·2$, $\beta_2 = 3·30$ (5) $\beta_1 = 0·5$, $\beta_2 = 3·73$. The sizes of samples were 2, 5, 10 and 20, respectively. As a result of two series of tests, *viz.*, the single and two sample tests, it has been shown that in the former case 21 tests of goodness of fit for *z* gave a mean value of 0·463 and in the latter case nine values of *P* gave a mean of 0·332. Pearson remarks that in these ranges of β_1 and β_2 'there will not in practice be a danger of any serious loss of control' with regard to the hypothesis being rejected when it is in fact true. It will be noticed that the observed values in the present series of field experiments mostly lie within the range covered by Pearson's work.

by twos have therefore been treated as blocks for eliminating soil heterogeneity and R. A. Fisher's method of analysis of variance has been used throughout for calculating experimental errors.

The residual standard deviations between plots are shown in Table II and the standard deviations expressed as percentages of mean yields in Table III for different shapes and sizes of plots for the year 1930-31. Corresponding figures for 1931-32 and for 1932-33 are given in Tables IV—VII.

TABLE II

Standard deviation of plots in tolas, 1930-31

Breadth (unit = 3·75 ft.)	(a) Before elimination of block differences			(b) After elimination of block differences	
	Length (unit = 72·5 feet)				
	1	2	4	1	2
1	30·17	42·46	54·27	21·66	33·75
2	58·33	81·98	103·20	40·68	63·86
4	112·04	156·21	189·00	75·19	117·71
5	139·41	196·71	239·71	93·53	149·43
10	262·09	368·30	405·10	162·09	262·65

TABLE III

Standard deviation expressed as percentage of mean, 1930-31

	(a) Before elimination of block differences			(b) After elimination of block differences	
	1	2	4	1	2
1	25·94	18·26	11·67	18·63	14·51
2	25·08	17·62	11·09	17·49	13·73
4	24·09	16·79	10·16	16·16	12·65
5	23·98	16·92	10·31	16·09	12·85
10	22·54	15·84	8·71	13·94	11·29

TABLE IV

Standard deviation of plots in tolas, 1931-32

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	23·74	35·38	55·13	19·76	30·90
2	45·16	67·74	105·2	36·96	58·52
4	86·78	130·97	202·1	70·48	112·47
5	106·54	161·80	250·6	86·33	139·00
10	197·79	311·75	485·3	168·23	269·62

TABLE V

Standard deviation expressed as precentage of mean, 1931-32

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	35·31	26·31	20·50	29·39	22·98
2	33·59	25·20	19·56	27·49	21·76
4	32·27	24·35	18·79	26·21	20·91
5	31·70	24·07	18·64	25·68	20·67
10	29·42	23·19	18·04	25·02	20·05

TABLE VI

Standard deviation of plots in tolas, 1932-33

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	32·26	50·36	49·21	29·83	46·95
2	57·31	95·15	86·44	52·10	88·30
4	108·76	185·87	171·3	99·31	173·66
5	136·86	230·69	191·6	126·44	216·52
10	260·34	448·50	393·1	250·11	432·86

TABLE VII

Standard deviation expressed as percentage of mean, 1932-33

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	25·53	19·93	9·74	23·61	18·58
2	22·68	18·82	8·55	20·61	17·47
4	21·52	18·39	8·47	19·65	17·18
5	21·66	18·25	7·58	20·01	17·13
10	20·60	17·74	7·78	19·79	17·13

It will be seen that the standard deviation does not increase proportionately to the increase of the size of the plot, in fact as the area of the individual plots is increased, the percentage standard deviation diminishes but this decrease is more rapid for plots of increased lengths than for increased breadths.

Thus, if unit of length (=72·5 feet) = l

and unit of breadth (= 3·75 feet) = b

we find the following results for 1930-31, without eliminating soil differences.

TABLE VIII

Standard deviations expressed as percentage per plot of different shapes

Size of plot	S. D.	Size of plot	S. D.
$(2l) \times (b)$	18·26	$(4l) \times (b)$	11·67
		$(2l) \times (2b)$	17·62
$(l) \times (2b)$	25·08	$(l) \times (4b)$	24·09

Thus, highly narrow strips of plots (length much greater than breadth) lead to greater precision than plots of same area but much wider and not so narrow. The same result has been obtained by a number of workers; Christidis [1931] has

shown on the basis of certain theoretical considerations, that plots should be as long and as narrow as possible in order to reduce the effect of soil heterogeneity; this " of course, within the limits set by different practical considerations, amongst which convenience, competition, and accurate measurement of width appear to be the most important ".

In the present investigation, the results of all the three years corroborate Christidis's conclusions, as can be seen from Tables III, V and VII for the years 1930-31, 1931-32 and 1932-33 respectively. The residual standard deviation has steadily decreased in all three seasons up to the largest area, for each individual width.

Soil heterogeneity

It is now generally accepted that soil heterogeneity is a characteristic of all fields and one of the objects of field designs is to eliminate, by local control, this source of variation as far as possible. It will be interesting therefore to investigate how far the precision of the experiment has been increased in the present design by using Fisher's method of Randomised blocks.

A comparison of the results given in the two portions.

(*a*) before elimination of block differences, and
(*b*) after elimination of block differences

in Tables II—VII will show the actual improvement achieved. Thus in 1930-31, for a plot size ($1l \times 10b$), the S. D. before block elimination is 262·09 tolas per plot while, after this elimination, this is reduced to 162·09 tolas per plot. It is true that the magnitude of the reduction is not the same for plots of all sizes, but practically in every case the block elimination has appreciably improved the precision of comparison.

We have seen that the percentage standard deviation decreases steadily as the size of the plot is increased. But for a given size of the experimental field, an increase in plot size also means a reduction in the number of replications. Thus while the error of the experiment is reduced by the decrease in the percentage standard deviation due to increase of plot size, it is at the same time increased by the loss in the number of replications.

It is clear therefore that in order to determine the optimum size of the plot, it is necessary to take both these factors into consideration. A suitable criterion is obtained by multiplying the percentage standard deviation of each plot size by the square root of the number of ultimate plot units which the plot contains. The final standard errors (corrected for plot size and number of replications) are shown in Tables IX—XI. These tables are now comparable for the effective precision of the experiment.

TABLE IX

Percentage standard error corrected for size of plot and number of replications, 1930-31

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	25·94	25·82	23·34	18.63	20.52
2	35·46	35·24	31·36	24.73	27.46
4	48·18	47·48	40·64	32.32	35.77
5	53·62	53·50	46·11	35.98	40.63
10	71·27	70·84	55·08	44.08	50.49

TABLE X

Percentage standard error corrected for size of plot and number of replications, 1931-32

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	35·31	37·20	41·00	29·39	32·49
2	47·50	50·40	55·32	38·87	43·52
4	64·54	68·86	75·16	52·42	59·13
5	70·88	76·11	83·36	57·42	65·36
10	93·03	103·71	114·08	79·11	89·66

TABLE XI

Percentage standard error corrected for size of plot and number of replications, 1932-33

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	25·53	28·18	19·48	23·61	26·27
2	32·07	37·64	24·18	29·14	34·94
4	43·04	52·01	33·88	39·30	48·59
5	48·43	57·71	33·90	44·74	54·17
10	65·14	79·33	49·20	62·58	76·61

For convenience of comparison, the effective standard errors are shown in Tables XII—XIV as percentages of the corresponding standard errors of the smallest plots.

TABLE XII

Standard error expressed as percentage of standard error of the smallest plot, 1930-31

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	100·00	99·54	88·98	71·82	79·10
2	136·70	135·85	120·89	95·33	105·86
4	185·73	183·04	156·67	124·59	137·89
5	206·71	206·24	177·75	138·70	156·63
10	274·75	273·09	212·33	169·93	194·64

TABLE XIII

Standard error expressed as percentage of standard error of the smallest plot, 1931-32

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	100·00	105·35	116·11	83·23	92·01
2	134·52	142·73	156·67	110·08	123·25
4	182·78	195·01	212·85	148·45	167·46
5	200·73	215·54	236·08	162·61	185·10
10	263·46	293·71	323·07	224·04	253·92

TABLE XIV

Standard error expressed as percentage of standard error of the smallest plot, 1932-33

	(*a*) Before elimination of block differences			(*b*) After elimination of block differences	
	1	2	4	1	2
1	100·00	110·37	76·30	92·48	102·90
2	125·62	147·43	94·71	114·14	136·86
4	168·59	203·72	132·71	153·94	190·32
5	189·70	226·05	132·78	175·24	212·18
10	255·15	310·73	192·71	245·12	300·08

Two things here deserve notice. The effective precision, as observed by previous workers, generally decreases as the plot size is increased. But this is not true invariably. We find in fact that, in the present series of experiments, a plot size of $4l \times 1b$ (290′ × 3′.75) in 1930-31 and 1932-33, $2l \times 1b$, (145′ 0×3′.75)

in 1931-32 give the greatest precision. It appears desirable therefore to use long plots of 1/40 acre running from east to west in future experiments with *juar* on this particular field. The next best size of plots will be 145′ × 3′.75 (1/80 acre).

Analysis of co-variance

In the foregoing analysis increased precision was obtained by block division. This procedure eliminates the soil-heterogeneity between blocks, but it is incapable of eliminating the soil differences existing between plots within the same block. Co-variance analysis is often of great help for this purpose where results for successive seasons are available, and has proved highly successful in the case of certain crops, *e.g.*, potato, sugar-beet, and tea.

In this method the correlation between the yields of two consecutive seasons is calculated, and if this correlation is statistically significant, the information for the first season is utilised for eliminating the fertility differences between plots within the same block in the second season. The improvement in precision increases naturally with the magnitude of the coefficient of correlation. In the present experiment however the residual correlations are practically negligible, as shown in Table XV.

Table XV

Coefficient of correlation

Year	Block	Residual
1930-31 × 1931-32	0·6250	0·1741
1931-32 × 1932-33	·9826	+0·0392
1930-31 × 1932-33	0·7516	—0·0536

The coefficient of correlation between blocks, being based on two degrees of freedom, may be considered significant only in the case of 1931-32 and 1932-33; the other two, though high, are less than the five per cent value. The correlation between plots is not appreciable in any of the three combinations. With such insignificant correlations it is not possible to improve the precision of the experiment in subsequent years with the help of the results of the previous seasons. It is however desirable to examine whether this is true for the *juar* fields in general.

Summary

Uniformity trials with *juar* (*Andropogon Sorghum*) were conducted for three successive years (1930-33) in Mohol plot in Sholapur district. A detailed statistical analysis gave the following results :—

(1) The frequency distribution of yields is not strictly normal ; the divergence from normality although statistically significant is not large.

(2) The frequency distributions were not significantly different from the normal type ; but as the sample was based on only 160 observations, this test of deviation from normality was not sufficiently precise.

(3) The systematic differences in soil-fertility were appreciably eliminated by dividing the field into two and four blocks, and the precision of the experiments was thereby improved very considerably.

(4) Long and narrow strips of plots gave greater precision than plots of same area but much wider.

(5) The standard deviation between plots decreased as the plot area increased, but the gain in precision was less than the loss due to the reduction in the number of replications.

(6) The effective precision (corrected for size of plots and number of replications) was highest in plots of 290 ft. × 3·75 ft. (or 1/40 acre). The next best plot was 145 ft. ×3·75 ft. (or 1/80 acre).

(7) The yield of plots within the same block did not show appreciable correlation from year to year, so that the method of analysis of co-variance failed to give increased precision in succeeding years.

Acknowledgments are due to Rao Saheb B. P. Wagholkar and Mr. V. V. Gadgil, Deputy Directors of Agriculture, for valuable help and encouragement and to Dr. W. Burns, Director of Agriculture, Bombay Presidency, under whose guidance the field experiments were conducted by one of us (Kulkarni). Our thanks are also due to Mr. Satyaprasanna Dev and Mr. Prabhatranjan Ray for computational help in the present analysis which was carried out in the Statistical Laboratory, Presidency College, Calcutta.

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Appendix 1

Sketch Map I, 1930-31

Yield of grain in "tolas" of original 160 plots of one acre during the year 1930

144	183	142	144
171	135	124	132
156	145	121	107
134	122	134	80
180	122	137	99
172	95	122	87
175	117	94	98
186	114	89	70
174	112	91	81
144	97	87	86
172	110	104	89
142	107	121	107
176	131	115	106
171	115	117	99
178	116	95	86
203	111	106	106
186	108	103	110
187	108	101	100
150	114	115	90
182	96	98	100
177	94	86	106
168	103	90	113
187	92	110	115
189	96	99	108
164	113	102	107
151	97	104	101
120	106	112	116
138	102	98	99
126	103	112	93
100	74	90	95
119	103	115	90
104	90	99	82
120	80	109	73
98	81	117	74
125	90	120	58
127	91	135	66
114	90	132	69
125	86	123	58
95	116	145	110
132	111	132	118

Sketch Map I, 1931-32

Yield of grain in "tolas" of original 160 plots of one acre during the year 1931

113	115	79	131
73	106	72	79
75	112	83	51
66	98	74	54
74	84	75	39
65	75	100	45
83	69	80	41
65	84	88	41
70	88	87	62
70	93	86	78
89	106	78	72
80	110	86	84
105	100	84	84
88	78	71	72
77	92	77	80
90	75	74	55
87	72	73	68
95	77	68	48
90	24	65	50
70	59	57	59
73	67	57	52
65	64	53	57
70	68	58	50
65	62	71	37
75	62	56	40
70	55	81	41
88	62	105	27
49	54	79	33
71	64	80	22
77	69	95	20
64	66	80	14
59	77	72	14
65	68	41	9
62	72	85	7
72	60	65	14
60	67	77	13
98	67	61	7
85	68	54	8
84	59	36	5
90	62	49	4

Sketch Map I, 1932-33

Yield of grain in "tolas" of original 160 plots of one acre during the year 1932

96	133	136	128
97	110	104	115
93	82	128	128
70	108	121	133
71	99	133	138
97	108	102	145
90	111	128	171
81	112	124	120
96	122	137	150
96	110	118	127
108	104	162	107
97	127	164	64
104	112	176	119
121	142	143	153
159	134	170	123
163	151	155	113
163	132	127	98
136	141	135	118
174	125	116	98
150	160	124	73
176	131	164	74
185	146	91	97
253	132	132	93
153	125	110	90
197	154	103	106
205	143	121	107
160	178	117	109
158	165	133	111
176	160	83	116
164	155	119	84
147	157	129	108
155	132	125	82
165	128	144	102
103	127	140	92
152	144	122	98
151	141	138	75
186	155	122	53
119	130	130	68
133	137	115	60
161	139	118	51

PRELIMINARY OBSERVATIONS ON SMALL-LEAF DISEASE IN COTTON

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(Received for publication 14th December 1935)

Introduction

A peculiar disease in cotton which manifests itself in the reduced size of leaves and sterility of the plant has since long been recorded in the Bombay Presidency. A few observations were made by Kottur and Patel [1920], but no attention appears to have been paid to this disease until 1933 when its incidence was reported to be as high as five to fifteen per cent in the Southern Maratha Country. In August of the same year, the Indian Central Cotton Committee provided funds, for a period of one year, for the survey of the disease in the Bombay Presidency including the Baroda State, but this work was not begun until very late in the season. Side by side with the survey work, preliminary experiments were made on the nature of the disease, but some of the experimental work was unfinished at the end of the year. The following report therefore is incomplete in some essential respects, but it has been considered worthwhile to record the observations made during the period of December 1933 to October 1934.

Distribution and extent of damage

A survey of the disease was undertaken in the important cotton-growing tracts of the Presidency, namely, Gujarat (including the Baroda State), the two districts of Khandesh and the Southern Maratha Country. In determining the amount of damage, an area of about four to five acres was selected at random in a village and the total number of plants in the whole area was calculated by multiplying the average number of plants in a line by the number of lines. The number of diseased plants was determined by actual count. From these figures the percentage of infection was calculated. However, when larger areas were selected for observation, the amount of damage was determined from the number of diseased plants in a few sections selected at random in the area. In addition, information on the following points was obtained whenever possible: (1) kind of soil, (2) variety of cotton, (3) rotation followed in the preceding years, (4) approximate date of sowing, and (5) insects found on diseased and healthy plants.

The incidence of the disease in each variety of cotton in the different tracts of the Presidency and in the Baroda State is summarised in Table I.

TABLE I

Amount of damage by Small-Leaf disease to different varieties of cotton

Variety of cotton	District	Percentage of diseased plants
	Southern Maratha Country	
Jaywant	Dharwar	0·1 —2·2
	Belgaum	0·24—2·3
	Bijapur	0·10—2·1
Kumpta	Dharwar	0·17—1·15
	Belgaum	0·10—1·70
	Bijapur	0·30—3·50
	Gujarat and Baroda	
Kanvi	Baroda	0·0 —1·18
	Mehsana	0·57—1·33
	Kaira	0·52—3·41
Desi cotton	Baroda	*Nil*
	Broach	0·05—0·5
	Surat	0·10
Rozi cotton	Kaira	*Nil*
Do. (1st ratoon)	Do.	8·75—13·94
Do. (2nd ratoon)	Do.	17·23—21·22 (even up to 33 per cent in some places)
B. D. 8	Surat	Less than 0·1
	Broach	0·0—0·2
Wagad local	Mehsana	4·88—6·74
	Ahmedabad	*Nil*
Wagad 8	Mehsana	0·40—9·39
Chokdio	Do.	0·55
Lalio	Do.	3·00
Broach 9	Baroda	0·10
1027 A. L. F.	Broach	Negligible
	Surat	Less than 0·1
	Khandesh	
Neglectum types	West Khandesh	*Nil*
	East Khandesh	*Nil*

It will be seen from the table that the disease is of widespread occurrence, but was not found in the southern part of the Surat district or in Khandesh. It was not severe in 1933-34, although its incidence ranged from five to fifteen per cent in the Southern Maratha Country in the previous year. Inquiries with the cultivators revealed that the disease varies in severity from year to year.

The survey in Gujarat was not undertaken until February 1934, when the cotton crop had been seriously affected by the severe frost, and it was difficult to recognise diseased from healthy plants in the field. In many places the picking of cotton was over when the fields were visited for observations, and it was further found on enquiry that cultivators in Gujarat uproot diseased plants as soon as they are noticed in the field and feed them to the cattle. The information on the amount of damage by the disease in Gujarat is therefore incomplete.

The survey work in Khandesh was begun in the middle of September 1934, and from the reports received till the close of the enquiry in October 1934, it was found that not a single diseased plant had been observed in the Neglectum types of cotton.

The following other points of interest are brought out by the table:

(*a*) The incidence of the disease varied from 0·2 to 3·5 per cent in the Southern Maratha Country and from 0 to 10 per cent in annual types of cotton in Gujarat, where the ratoon crop usually suffers damage up to about thirty-three per cent. In Khandesh the disease was not observed. On the whole the disease was much less in evidence in 1933-34 than in the previous year.

(*b*) In the Kaira district, where ratooning of cotton is normally practised, the disease appears in a virulent form in ratoons of Rozi cotton. The first year's crop is free from the disease, which appears in the ratoon crop to the extent of ten to fifteen per cent, and is greatly intensified in the second ratoon, the damage varying from eighteen to thirty-five per cent.

(*c*) Foreign varieties and the Neglectum types of indigenous cottons were found to be free from the disease. All Herbaceum types were affected.

(*d*) There was no evidence that any variety of Herbaceum cotton was more resistant to the disease than another.

On the Dharwar Farm, observations were made on the appearance of the disease in cotton in an area of $1\frac{1}{2}$ acres. Of the total number of diseased plants observed from November 1933 to February 1934, thirty-six per cent were noticed in November, 42·8 per cent in December, seventeen per cent in January and four per cent in February. The disease continued to appear even after February, but late attack did not affect the yield as the bolls were fully opened by that time.

No survey was made in the Ahmedabad and Panchmahal districts, and in the Navasari district of Baroda State.

Symptoms of disease

Before the appearance of the disease, the leaves on all plants are alike in colour and size, and no distinction can be made between a diseased and a healthy plant. About twelve to thirteen weeks after sowing (or sixteen weeks in the late-sown crop), a plant here and a plant there in a field may be seen putting forth numerous small leaves in clusters, and the dormant buds on the main stem as well as the axillary buds are stimulated to activity, thus indicating distinct departures from normal growth. The leaves on diseased plants are considerably reduced in size and measure from 0·3 to 2·3 cm. in length and 0·5 to 3 cm. in breadth, whilst those on healthy plants measure from 1·4 to 3·4 cm. in length and 1·5 to 4·1 cm. in breadth. The colour of diseased plants gradually changes to pale green, and old leaves on such plants, though of normal size, turn red along the margins on the upper or the lower surface. The reddening continues in the tissue between the veins, and such leaves wither and drop down in about tree weeks. On the small leaves', however, small black specks appear, but these leaves may or may not turn red along the margins. The diseased plants give the appearance of a "witches broom". No mottling of the leaves has been observed, although curling is present to some extent.

The petioles are reduced proportionately in size. The bracteoles attain a good size, and persist on the plant for a long time, but the bud inside the bracteoles becomes flat and suppressed and withers in a few days. Over the suppressed bud the calyx forms a cup-like organ. The petals are changed into small, thick, leafy structures, and other floral organs are extremely reduced in size. Such plants remain sterile.

An affected plant has never been seen to recover, and after the appearance of the disease its rate of growth is much slower than that of a healthy plant. The diseased plants wither within the course of two to three months. Many plants are partially affected, the healthy part producing normal bolls. If a plant is affected after the bolls are formed, the bolls do not make much further growth but are cracked and open out early. The disease may originate in any part of the field and not necessarily along the borders. There is no marked difference between the root systems of the diseased and healthy plants. It is not usual to find all the plants in a hill showing the disease.

Nature of the disease

That the disease is not parasitic in nature has been proved by repeated isolations from the affected plants. There is also no evidence that atmospheric or soil conditions cause the disease. Analyses of soil samples from around the diseased and healthy plants have shown no difference in their chemical composition, nor has the application of farmyard manure in varying quantities to different plots pre-disposed the plants to infection.

None of the cotton plants inoculated with the juice of diseased plants showed symptoms of Small-Leaf disease. Also, all attempts to transmit the disease by means of insects from naturally infected cotton plants to healthy plants in muslin cages gave negative results. The result of the transmission of the

disease by grafting were inconclusive, since these experiments failed due to graft union not being successful. There was no opportunity to repeat these experiments.

Study of the disease in the ratoon crop

Field observations indicate that ratoon cotton suffers badly from the disease. The following is the brief summary of the observations made at different places.

Surat Farm.—Five hundred and seventy-six healthy plants of 1027 A.L.F. were cut back in May 1934. About the end of September, 1·2 per cent of these plants showed definite symptoms of Small-Leaf disease.

Broach Farm.—Two hundred and fifty-four healthy plants of B.D.8 cotton were ratooned and 1·2 per cent of these were infected.

Lambhvel and Bhumel (Kaira district).—No disease was observed in Rozi cotton in the first year. In the second year crop, *i.e.*, the first ratoon, 3·26 per cent plants were diseased out of 675 plants examined; whilst in the second ratoon that attack was greatly intensified, about 35·7 per cent of 1,120 plants examined showing infection.

Village Jamania in the Surat district.—An area of about seven acres under 1027 A.L.F. was examined, but only one diseased plant was observed, although cotton is commonly grown as a ratoon crop in this tract. It appears that the ratoon crop of Rozi cotton in the Kaira district is more liable to disease than 1027 A.L.F.

Dharwar Farm.—After uprooting all diseased plants, the apparently healthy plants were cut back on the 12th June 1934, leaving about six inches of stem above-ground. Most of the plants sprouted as there was good rain in the month of June. Whilst many plants put forth normal leaves and branches, the leaves on some of them were very small, irregular in shape and much crowded near the sprouting buds; the latter plants did not make much growth but for want of definite symptoms, they could not, however, be classified as diseased. About two months after sprouting, the leaves on these suspected plants began to wither, and by the middle of September, twenty-four out of 303 plants under observation showed definite symptoms of Small-Leaf disease. Of these twenty-four plants, four were stunted in growth from the beginning, and the remaining twenty plants became infected later in the season. Unfortunately, however, both the healthy and diseased plants withered due to lack of rains, and no further observations could be made.

Gadag Farm.—The cotton plants were cut back on the 25th May 1934. Out of 378 plants, 257 plants sprouted, but all withered due to lack of rains.

Conclusions

It may be noted that, besides ascertaining the extent and the amount of damage caused by the disease, one object of the survey was to accumulate preliminary evidence relating to the nature of the disease. The information collected during the year, though of a preliminary nature, points to the disease being a teratological malformation.

SUMMARY

The disease is of widespread occurrence and affects varieties of cotton of the Herbaceum type; none of the Neglectum types has been found to be infected. The disease does not attack foreign varieties of cotton.

The disease was much less in evidence during the year under report. The severity of attack varies from year to year.

In the Kaira district the disease is serious in the first and second ratoons, although the crop in the first year is free from it. The damage to the second ratoon may be as high as thirty-five per cent.

The symptoms of the disease have been described. The important symptom is the reduced size of the leaves which appear in clusters. The affected plants are sterile, and the floral organs show teratological malformations.

No evidence has been obtained that parasitic organisms are associated with the diseased plants; nor does the disease appear to be caused by insects or by soil or climatic conditions. The disease was not transmitted to healthy plants by insects taken from naturally infected plants nor by the juice expressed from the diseased plants. Experiments on the transmission of the disease by grafting were inconclusive.

Observations on the disease in ratoon cotton at various places have been summarised. It appears that the Rozi cotton is more liable to disease as a ratoon crop.

The writer is grateful to the Indian Central Cotton Committee for the provision of funds which enabled him to do this work.

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INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET

VIII.—A BROWNISH PURPLE MUTANT

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(With Plate XXXII)

The prominent vegetative part of the sorghum plant is usually devoid of marked purple pigmentation. In red-grained varieties there is a reddish sap colour that appears mostly at the margins of the leaf, when it begins to dry up. Apart from this colour, sorghum does not show much distinguishing purple pigment in the vegetative parts. Such colour differences as there are seen only in the grains and in the dry anthers. Ther eis thus an absence of noticeable purple pigmentation in the vegetative parts. This pigmentation is however potential and is activated on mechanical injury. This pigment is anthocyanic and does not colour the mechanical tissue.

Since the inception of the intensive study of sorghums in Madras in 1923, millions of individual plants from many varieties have been marshalled for examination and till the year 1931 no instances have been met with of a plant with internodes brownish purple and the midribs coloured likewise. This brownish purple was not anthocyanic, and seems to be somewhat similar to the brown of the brown midrib in maize. [Jorgenson, 1931, and Burnham and Brink, 1932].

In the variety of *Periamanjal cholam*, the prominent grain sorghum variety of Coimbatore, intensive selection work has been in progress for a number of years. Among the selections of this variety, which is green-stemmed and whose leaves have a white midrib, there occurred in 1931, a brownish purple mutant. This mutant, while being very slightly shorter and weaker, was in general configuration like the rest of the population, only it had brownish purple-lined internodes and leaf-sheaths, and brownish purple midribs in the leaves. The

internode colour was present even on the portions of the internode covered by the leaf-sheath. This plant bred true. Crosses between this mutant and a plant of the mother population showed that the colourless internode and midrib were dominant, setting down this brownish purple mutant as a definite recessive. The second generation plants gave the following segregations.

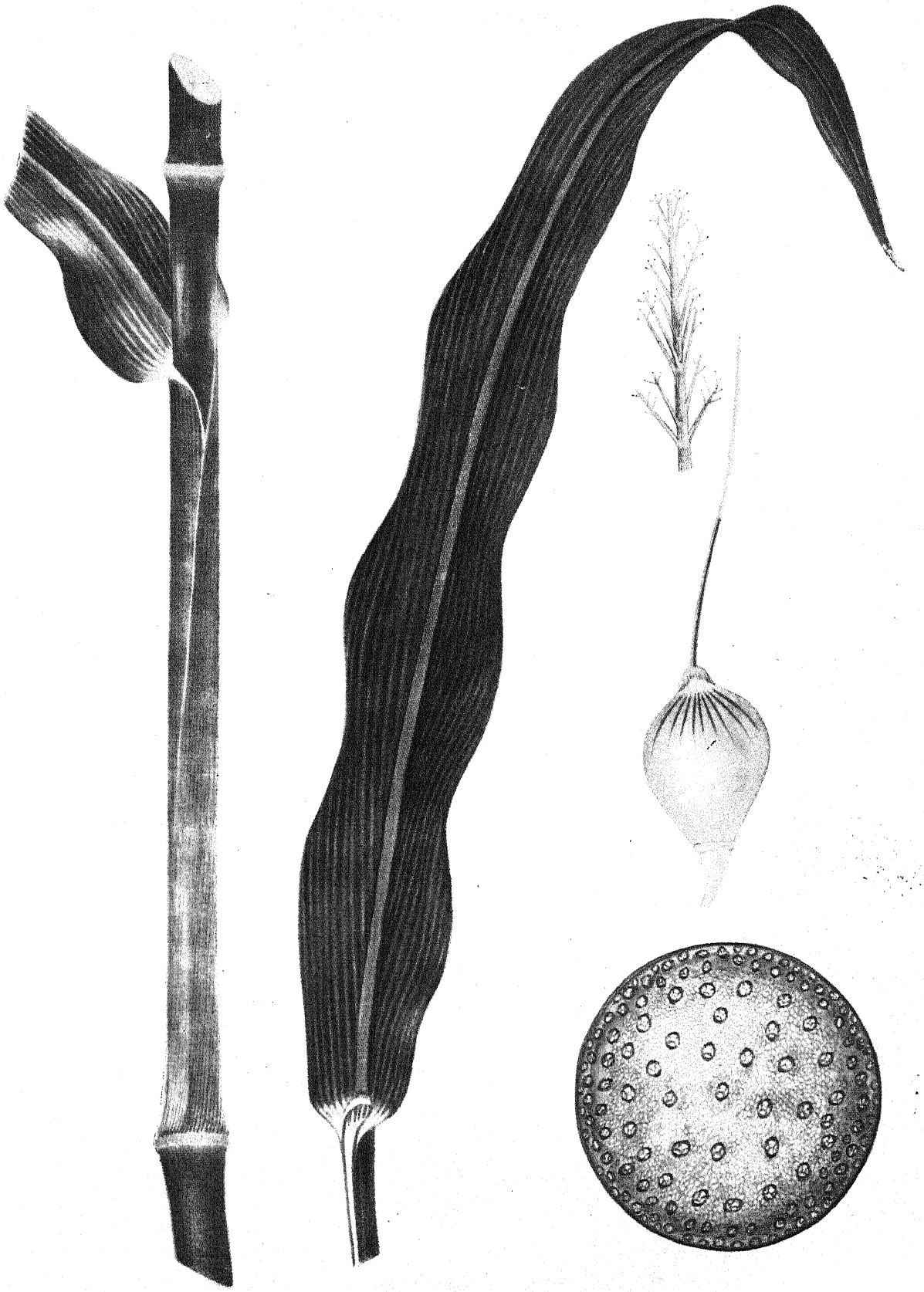
Family No.	F_2 segregating for	
	Green internode and leaf-sheath, white midrib, etc.	Brownish purple lined internode, leaf-sheath, midrib, etc.
A. S. 3636	121	37
„ 3645	127	41
Total (observed) . .	248	78
„ (calculated) .	245	81

$\chi^2 = 0 \cdot 15$, $P > 0 \cdot 5$

It will be seen that the segregation is monofactorial and that the mutant has proved a simple recessive.

Sections of the stem, exposed roots and panicle branches show that the pigment colours the mechanical tissue of the plant (Plate XXXII). It may be noted that the coloured stems were a bit harder to section.

The first traces of this brownish purple pigmentation are noticed when the seedlings are even a week old. The midrib is the earliest to develop the colour. The upper surface of the midrib is coloured brownish purple, while the lower surface is more deeply coloured, with an accentuation towards the ridge of the midrib. The intensity of colour in the midrib increases with the growth of the seedlings, and in about thirty to forty days after sowing the leaf-sheath also begins to manifest the pigmentation in lines. In due course the other parts of the plant, *viz.*, internode, panicle branches, glumes and even the exposed roots develop the colour. It is during the milky stage of the head that the deepest manifestation is noticed and at this period the stem, exposed roots and glume nerves are of a good brownish purple colour. After the milky stage there is a gradual falling-off of the colour, first in the midrib, then in the leaf-sheath, panicle branches and glume nerves. In the midrib a faint brownish purple over a yellowish background is retained to the last. The colour in the internode remains even after harvest, but gradually fades into a pale brownish purple tint in the stripped dry stalks.



Colouring effect of pigment on the mechanical tissue of the plant.

The non-existence of this brownish purple type in current varieties and this rare occurrence as a recessive mutant connoted probable disabilities. Over 300 plants in each of green and brownish purple internodes were examined and the following notes recorded:—

	Green	Brownish purple
Average height of plant	320 cm.	270 cm.
Average weight of earhead	80 grms.	70 grms.
Average weight of straw	330 grms.	250 grms.

In segregating families this difference tended to be kept up, the disabilities of the brownish purple going with it. The brownish purple plants were more juicy than the green ones at about the milky stage. Later on they dried up more rapidly than the greens and left lighter stalks.

Sorghum varieties vary in their juiciness, height, weight of earheads and other economic characters. The particular disabilities of this brownish purple mutant are therefore by themselves not likely to have been the deciding factors in the rarity of their occurrence. This mutation in sorghum may therefore be an echo of an ancestral equipment. Inhibitory factors with favourable physiologic reactions have probably operated to suppress this pigmentation and create the wide group of grain sorghums devoid of brownish pigmentation in their mechanical tissues. A factor $\mathbf{mt_b}$ gives a mechanical tissue coloured brownish purple. $\mathbf{Mt_B}$ gives the ordinary green internode of the common sorghums.

Summary

The occurrence, as a mutant, of a sorghum plant with brownish purple-lined internode, leaf-sheath, midrib, panicle branch, and glume top is recorded. The pigment is not anthocyanic. This mutant behaves as a simple recessive to the common more economic green-internoded and white-midribbed plant. A factor $\mathbf{mt_b}$ gives a mechanical tissue coloured brownish purple. $\mathbf{Mt_B}$ gives the ordinary green internode of the common sorghum.

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BANANA BREEDING AT THE IMPERIAL COLLEGE OF TROPICAL AGRICULTURE, TRINIDAD

(With a view to obtain a type resistent to the Panama disease, includin genetical and cytological studies on allied species)

(*Summary of work done by Prof.* E. E. CHEESMAN *and Mr.* L. N. H. LARTER, *as reported in the Empire Marketing Board's Report of December, 1931, and Journal of Genetics, Vol. 26, No. 3, December, 1932, and Vol. 30, No. 1, January, 1935*)

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Professor E. E. Cheesman of the Imperial College of Tropical Agriculture, Trinidad, has carried out very valuable work in the production of a new variety of banana, combining resistance to Panama disease with the good commercial qualities of Gros Michel, the present outstanding variety of northern markets. The following lines are a summary of work done by him, and the results obtained upto the year 1935.

The importance of banana as a dessert fruit is emphasised by Dr. T. Drummond Shiels [1931], Chairman of the Research Grants Committee of the Empire Marketing Board, in the following words: "Fruit is steadily becoming more popular in the United Kingdom, and the banana is fully sharing in this very welcome trend of public taste. A record for the consumption of bananas was set up in 1930 by an import figure of almost 15,000,000 bunches. Nearly 6,000,000 of these came from the West Indies, whose exports of bananas to the Home market have increased very rapidly in the last five years. The banana industry, therefore, is important both to this country which is concerned with securing an ample supply of good fruit from Empire sources, and to parts of the West Indies, which are depending more and more on the crop for their living".

The work detailed here is considered to be of immense value to the banana growers of India also. The importance of the banana crop to this country may be easily gauged from the fact that the Presidencies of Madras and Bombay alone grow 144,000 and 21,556 acres of banana, respectively, with a very wide field for further extension. At the same time, the Panama disease has been recorded in several places in this country, and many flourishing plantations are seen to be devastated by it within a very short period. The account of work done at Trinidad, therefore, will be found highly valuable to the Indian growers and fruit merchants at this juncture.

This summary is divided into three sections, the first dealing with the Breeding Work. The second and third sections deal with cytological studies of bananas and allied species carried out with a view to elucidate their breeding behaviour. Incidentally, the cytological work has thrown considerable light on the internal classification of the Sub-order, *Musaceae*.

Section I

Banana breeding at the Imperial College of Tropical Agriculture, Trinidad [Cheesman; 1931, 1].

At the Imperial College of Tropical Agriculture, Trinidad, the banana breeding work has been in progress since 1922. The Gros Michel banana combines a variety of commercially desirable characters, but it is highly susceptible to the Panama disease. Several other varieties possess the character of being immune to the Panama disease, but they lack in commercial qualities. The "building in" together of these two sets of characters by breeding has presented a good deal of difficulty in view of the facts:—

(1) that the commercial banana is ordinarily a sterile type producing no seeds,

(2) that it is essential to induce seeds in it for any breeding work to be possible at all,

and (3) that the seed-bearing character should be finally eliminated in any new type, which may be evolved, before it can be commercially unblemished and popular.

The difficulty is further accentuated by the fact that the bananas of the world have not been properly studied and classified, and the present knowledge about them is imperfect.

Previous investigations

The work done by Tischler [1910], D'Angremond [1912, 1914], White [1928] and others has shown that the pollen formation in some varieties like Dole (8 chromosomes) is regular, while it is very irregular in others like Pisang Klady (16 chromosomes), Radjah Siam (24 chromosomes), etc., the irregularity apparently increasing with the increased number of chromosomes. Tischler [1912] found that the development of embryo-sac was normal in fertile and abnormal in sterile types of bananas. He recorded that in Java certain edible

types of bananas often produced seeds. D'Angremond obtained a few seeds in Gros Michel and Appelbacove varieties, and found pollen development in Gros Michel as highly irregular. The fertile varieties he studied were more regular in this respect. The work of Palm [1920] suggests similar behaviour in other plants belonging to *Musaceae* and *Zingiberaceae*, indicating that this type of irregularity exists in the whole order of *Scitamineae*.

White [1928], who chiefly used root tips for his cytological studies of bananas, observed an excellent polyploid series in about 150 varieties of *Musa*, and held that the basic haploid number of chromosomes in bananas is 4. White further recorded that the series is not quite euploid, counts of 18, 22, 23 chromosomes also having been obtained.

The F_1 generation produced from a cross made by D'Angremond [1926] between two fertile species showed that the reduction of chromosomes is very irregular and that the hybrid produced little fertile pollen. He observed that increased pollen fertility was a feature of the back-cross progeny, no individual of which exhibited parthenocarpy.

Details of investigations

The banana breeding work at Trinidad was the result of the initiative taken by the Colonial Research Committee, who were alarmed by the spread of the Panama disease in Central America. Sir Francis Watts, then the Imperial Commissioner, proposed an extensive collection and study of banana varieties at a central station, as in the case of sugarcane, in order to combine the commercial qualities of Gros Michel with resistance to Panama disease. In the year 1922, the Imperial College of Tropical Agriculture was founded at Trinidad, and this work was started there with the financial aid of the Colonial Office.

Facilities were offered by the Trinidad Department of Agriculture to work upon six varieties of bananas to start with at the St. Augustine Experiment Station. Dr. T. G. Mason and Professor J. S. Dash worked successively in the earlier stages of this research, and began investigations with a very large-scale pollination experiment. Several edible varieties of bananas were inter-crossed, as well as crossed with fertile pollen of seed-bearing types, namely *Musa ornata*, and *Musa malaccensis*.

Before proceeding further, it would be proper here to describe the main characters of the two parental types of bananas, Gros Michel (female parent) and *Musa malaccensis* (male parent), which have played important roles in these investigations.

The Gros Michel is a well-known commercial variety of banana from West Indies, tall and vigorously growing in habit. The fruit bunch hangs vertically downwards, and is very compact fulfilling commercial requirements. The fruit is about six inches long and tapers towards the end. When ripe, all the angles on the fruit get rounded, and a bright golden yellow colour is developed. This variety is, however, highly susceptible to the Panama disease. Owing to its popularity, commercial importance and breeding potentialities, it will be noticed in the following account that much attention has been devoted to it.

The seeded variety from which pollen were obtained to cross on Gros Michel was *Musa malaccensis*, the origin of which is not definitely known. It seems to be a variety of *M. malaccensis* Ridl., which latter is itself according to Backer, a variety of *Musa zebrina* Van Houtte. Several authors consider *M. zebrina* and *M. malaccensis* as synonyms, and the former name, having the advantage of priority, should be adopted. The plant is similar in habit as edible bananas, except that it is less vigorous in growth. The fruit bunch is irregular and is held at an angle above the horizontal. The fruit is about three inches long, round and has a pronounced beak. The ripe fruit has a golden yellow colour. Seeds are abundant. No case of parthenocarpy is yet observed in the fertile species of Musa.

As a result of cross-pollination, forty-nine perfect seeds in Gross Michel, and seventy-four in Silk, were obtained with pollens of the seeded types, except one seed which was obtained from a cross of Gros Michel by the edible type Congo. Of the Gros Michel lot, seventeen seeds germinated and only five seedlings survived to fruit.

Side by side with breeding work, botanical and mycological studies and tests of resistance of varieties were also carried on separately by Professor Dash and Professor Ashby. Dr. S. C. Harland and Mr. Cheesman took over the breeding work in 1923.

In March, 1925, one of the Gros Michel hybrid seedlings flowered and produced a fair-sized seedless fruit of good flavour. This seedling was then labelled GM×B2. This plant resisted Panama disease during six years of severe test, and was re-named I. C. 1 (Imperial College 1). It had, however, some commercial disadvantages, and an occasional seed appeared in its fruit as a result of self-pollination, when it was propagated by suckers, and grown under ordinary banana field conditions. Further work was continued to improve upon I.C.1. Cytological studies of the hybrids and their parents were also commenced at this stage, and extensive pollinations were made on a number of varieties. I. C. 1 was further multiplied and back-crossed to each of its parents.

As a result of Dr. Harland's visit in 1925, to several Tropical countries of the world, many new types of banana have been introduced into Trinidad to work upon.

After several unpromising attempts with other varieties of bananas, crossing and back-crossing on Gros Michel were resumed in 1927, and continued since then. Back-crossing of I. C. 1 on Gross Michel was for some time concentrated upon as promising a type, more akin to Gros Michel but quite resistent to Panama Disease. But these two types proved to be cross-sterile. Cytological studies, however, revealed that the I.C.1 seedling possesses a double complement of Gros Michel chromosomes, and as the results of back-crossing were considered uncertain, I.C.1 was left as it is. The breeding problem was tackled from other sides of attack.

Since it is held that the present edible types of bananas must have originated from wild seed-bearing types, a general survey of the genus *Musa* was considered valuable in this work. Examination of the recorded systematic

knowledge of *Musa* was, therefore, taken up. It was found that 134 specific names must have been listed under *Musa*. A much larger number of varietal names were found under *Musa sapientum*. The numerous languages and dialects of the world seemed to increase the number of banana varieties almost indefinitely, although many of them may be identical types. It was, therefore, considered necessary to ignore a large section which was useless for the problem in hand, and basing the studies on the *Eumusa* and *Rhodochlamys* groups, twenty seeded types and forty edible cultivated types have been collected at the Imperial College of Tropical Agriculture, Trinidad. In the elimination of identical types, rigid tests, including cool storage qualities were adopted. By this method of comparison and elimination, nearly a hundred original types are now reduced to less than thirty "Standard types" for basing future work upon.

The technique of introducing these varieties has been necessarily elaborate, in order to minimise the import of new diseases to the central place of collection along with suckers of the varieties. All introductions were certified as healthy in countries of origin, then first taken to Kew, where a special quarantine was built for this purpose. When they produced new suckers, which were considered healthy under the Kew quarantine conditions, the suckers were sent to Trinidad, where again they were further tested in an insect-proof greenhouse, before planting in the open varietal plot. This course was essential in the case of sterile varieties, which had to be introduced by means of vegetative suckers. In the case of seeded varieties, the question was much simpler, although seeds had to be obtained with considerable difficulty and risk from unexplored wild regions.

The details of actual breeding work carried on at Trinidad can best be summarised under three headings, namely,

(1) breeding from Gros Michel,

(2) breeding from other edible varieties,

and (3) breeding from seeded types alone.

General summary of breeding work

Technique.—The technique of the operations in the breeding work consisted of bagging inflorescences of both the male and female parents with calico bags before the bracts lifted in order to keep up their purity. Pollination was done daily in the morning on inflorescences which had just opened. A scalpel or camel hair brush, duly sterilised in alcohol after every change of pollen, was used for transferring pollen. Labelling was carefully done in each case, giving all necessary details such as pollen used, date of pollinating, etc. The bunches which formed were harvested when fully developed, and collected in a separate room for searching seeds in their fruits. Seeds of edible varieties were carefully sown individually in pots, and seedlings were, after proper hardening, transferred to the nursery. Only suckers from nursery stools were planted out in the field for further observations keeping back enough material for cases of emergency.

Sterility in bananas.—Sterility in bananas is manifested in their poor seed-producing capacity as well as in the low germination of seeds. Seeds are often produced without endosperm. Even after germination, abnormal growth or lack of vigour results in premature death of the seedlings. From 1,000 inflorescences pollinated, hardly 474 seeds were produced, of which only 42 germinated.

Breeding from Gros Michel

Breeding from Gros Michel is being explored in every direction, as its potentialities are vast. The I.C.1 seedling is not yet considered the best that could be obtained. In crossing Gros Michel, both edible varieties which produce fairly abundant pollen, as well as fully fertile species are used as male parents. As stated above, Gros Michel and I.C.1 presented cross-sterility. The pollen of Gros Michel itself is also being used on other seeded types. A second immune seedling I.C.2 from Gros Michel crossing was also used for taking pollen but it gave no result.

Attempt is made to produce the F'_2 generation of the I.C.1 type both by artificial and natural pollination, and nineteen seeds thus produced have yielded three seedlings for further study. Back-crossing of I.C.1 on its male parent resulted in abundant seeds, but not in a single economically useful seedling.

Breeding from edible varieties other than Gros Michel

In breeding from Gross Michel, attempt was made to combine disease resistance with its good characters. But in breeding from other edible types, which show resistance to Panama disease, the difficulty is accentuated by their undesirable commercial characters. Such characters would be required to be eliminated. From the breeder's point of view, however, the preliminary questions would be to find out the seed-producing capabilities and the kinds of offsprings each type might give. Work was commenced with a view to solve these two points on Cavendish, Congo, Sapientum, and Fillbasket group of bananas.

Plants of *Musa cavendishii*, known as the Cavendish, Canery or Chinese banana, have been introduced from various parts of the world to study the variability within its sub-groups. The fruit of this type is of high quality. The chances of breeding from this group seem to be slender. No seeds are yet obtained although several attempts at crossing were made.

The Congo group includes five varieties of dwarf plants. They are very similar to Gros Michel, being marked off from it by their resistance to Panama disease, cold storage qualities and breeding behaviour. Over 140 attempts to cross this group with several other seeded and unseeded types resulted in a single seed being obtained.

Musa sapientum includes a great variety of edible bananas. They are in general of poor dessert quality. The pathology of this group is not exhaustively studied, but some of them seem to be resistant to Panama disease. These

varieties are studied chiefly for genetic information, and their behaviour is interesting. They produce a large number of seeds, most of the seeds being empty. Seedlings from a cross with a member of this group proved to be intermediate between the parents, showing no doubling of chromosomes from the female parent as in the case of Gros Michel.

The Fillbasket group includes the Mysore type and has a few characters, which recall *sapientum*. The Fillbasket has resistance to Panama disease and wind damage; it has a vigorous habit with neat and compact bunch, good fruit colour, and fairly abundant seeds on pollination. But the fruits are small and thin-skinned. Out of fourteen germinations obtained from about 200 seeds got by crossing this group, eight robust plants of promise are growing in the nursery at the Imperial College (1932).

Pollinations on miscellaneous edible varieties have brought out the facts that—

(1) generally seeds are more common in red varieties,

(2) a yellow-fruited variety may often arise from red types,

(3) Coccenia (not related with *M. coccenia* Andr.) is a seed-producing resistant banana with promise.

A few pollinations on plantains have yielded two seeds, plants from which were studied.

Breeding from seeded types alone

A large number of seedlings with "intermediate" characters were obtained by breeding from seeded types alone. These types also are generally self-sterile. Back-crosses on the parents of the hybrids are more successful and are seen to produce uniform progeny suggesting that the chromosome complements go over without reduction. D'Angremond's [1926] trials, supported by the Imperial College results, show the possibility of evolving edible bananas which usually have a chromosome number of 33, from seeded types which have 22 chromosomes, by raising species-crosses having 33 chromosomes. The great requirement is to find the proper parent species for this end, and an enormous amount of work will have to be done by making hundreds of crosses and back-crosses in future.

Section II

GENETICAL AND CYTOLOGICAL STUDIES OF MUSA

1. Certain hybrids of the Gros Michel banana [Cheesman, 1932]

In the efforts to evolve a disease-resistant and commercially unblemished type of bananas, cytological study of banana varieties was considered very important to elucidate the breeding behaviour in the parthenocarpic forms.

Professor Cheesman's [1932] investigations detailed here do not support White's [1928] theory that the basic haploid chromosome number of the banana complex is 4 (*vide* page 486), and that the common condition in edible bananas is octoploid, but they indicate that the basic haploid number is 11, and the common condition is triploid.

Professor Cheesman's cytological investigations [1932] have been confined to somatic divisions and meiotic divisions in pollen mother-cells. The terminal bud of banana inflorescence, after it has passed the "female" (or fruit-bearing) stage, was cut and removed at once to the laboratory. The bracts with the "hands" of flowers were dissected and kept separately. The likely flower was crushed in a drop of water, and examined under a low power microscope for the condition of the pollen grains. Flowers at the stage when they could be examined measured about one cm. long and two mm. in diameter. They presented no difficulty in fixing. Root tips for examination were also readily obtained from rhizomes kept in a moist atmosphere in the dark, or planted in sand. The time between 5 and 6 o'clock in the evening was found best to fix the material. Desirable stages were almost invariably found at this time, although several fixations were necessary, before satisfactory preparations could be obtained. The most satisfactory fixative was found to be Tellyesnicky's Bichromate acetic acid mixture (six per cent potassium bichromate and ten per cent acetic acid). After washing the material was treated by a modification of Larbaud's [1921] method, and sections were cut at 5 μ and 10 μ and stained in Newton's Gentian violet. Euparal was preferred for mounting.

Cytological studies of Gros Michel, the female parent of I. C. 1

D'Angremond [1912, 1914] and White [1928] studied the cytology of Gros Michel and they both agree that its diploid chromosome number is 32. But the number found by Cheesman [1932] based on counts made at the first meiotic division and in root tips is 33.

In D'Angremond's [1912, 1914] studies, the chromatic substance of the nucleus was in the form of larger and smaller grains mostly lining the periphery. He never found a typical diakinesis stage and found the anaphase with the chromosomes straggling on the spindle.

White [1928], also found the chromatic material near the periphery, and he describes them as definitely 32, and in many cases pairing. He states that diakinesis is rather brief, and at this stage, he holds, that there are eight unpaired chromosomes and twelve pairs. At anaphase stage, White states that twelve chromosomes pass early to the poles, while eight lag near the equator showing a longitudinal split.

Cheesman's [1932] studies of Gros Michel show that the earliest stages show a loosening synizetic knot having threads of the bead string structure. Usually several round chromatic bodies and the nucleolus are present. In the next stage, long loops, characteristic of diplotene, are formed and the conjugation is more than simple pairing.

Diakinesis was observed in several cases, but it was difficult to fix this stage. The threads break into round granules connected in rows, and the chromosomes were actually shortening and thickening as usual.

The metaphase stage has various associations of which trivalents are more common. Two-thirds of the complements seem to be more commonly grouped than the remaining one-third. A very crowded and narrow equatorial plate is also formed in due course. Occasionally chromosomes are seen widely scattered, and perhaps in such cases the plate is omitted. In the typical anaphase stage, it is seen, that eleven chromosomes aggregate towards each pole, leaving eleven lagging of which several show signs of dividing.

After division I, the wall or walls rarely separate simple nuclei. Lagging chromosomes either remain isolated, or join several together to form secondary nuclear bodies. An irregular second division follows, and walls then form in every direction. This gives rise to multicellular "tetrads".

The number of pollen grains is small, their size variable and they are mostly empty.

Cytological studies of Musa malaccensis, *the male parent of I. C. 1*

The type "Seeded B", as *Musa malaccensis* was labelled at the Imperial College collection, showed no indication of segregation or abnormality of any kind when tested for its genotype. This type has 22 somatic chromosomes. Meiosis in the pollen mother-cell is not completely regular, one or two pairs of chromosomes lagging on the spindle. At metaphase of the first division, the chromosomes form a compact plate, and lend to counting only as they separate. The actual type of association seems to be five bivalents and three quadrivalents, or six bivalents grouping into three pairs, and five remaining more widely spaced.

The association persists into late anaphase, when the chromosomes show the split of the approaching second division, and reappears at the homotype metaphase. The tetrads regularly consist of four cells (very rarely five cells), but vary in their arrangements. The pollen is highly uniform in size, and about 100 μ in diameter. It germinates and grows well on one per cent agar with thirty per cent cane sugar, and 0·02 per cent citric acid. The percentage of germination in culture has not exceeded fifty. Seeds also have low germination power. These facts do indicate a certain degree of sterility in the seeded types of bananas also.

Cytological studies of the F_1 *generation, I. C. 1*

The four original hybrids in the F_1 generation closely resembled the female parent, Gros Michel, and were alike among themselves except that physiologically I. C. 1 alone was resistant to the Panama disease. The bunch of I. C. 1 differs from Gros Michel in hanging slightly less vertically and

being less compact. The fruit is rounded at the apex. The size and colour of the fruit, thickness of skin, terminal bud and male flowers are closely similar to those of Gros Michel. The male flowers of I. C. 1, however, produce abundant fertile pollen, but the production of seeds is scanty. Fruits develop parthenocarpically, and are edible and of good flavour.

The somatic chromosome number of I. C. 1 is 44, and on the assumption that this represents thirty-three from the female parent *plus* a haploid set from the male, the close morphological resemblance of Gros Michel becomes evident. The meiotic divisions are slightly more regular than those of Gros Michel, but the spindles are more compact and crowded. The chromosomes at metaphase of the first division are grouped into some associations of high valency which are difficult to analyse.

At anaphase, often both lagging and scattered chromosomes occur. Types of "dyads" are commonly met with during the second division. Lagging chromosomes are cut off in wedge-shaped cells, while scattered ones are distally severed. A proportion have only one cell surviving while the rest are disorganised. The whole set occasionally undergoes necrosis.

As in Gros Michel, multicellular "tetrads" are formed. Ripe pollen grains are irregular in size, though more abundant. Viable pollen grains carry 22 chromosomes.

The sister plants of I. C. 1 also gave similar results so far as they were examined. Among F_1 plants produced from later pollinations, two have entirely differed from I. C. 1, suggesting chromosome incompatibility by their very appearance. One of these plants was very dwarf, but both of them showed 75 chromosomes. Counts varied from cell to cell, and it may be that the true count is 77 (which is a multiple of the original basic haploid 11).

Cytological studies of the F generation

In the F_2 generation, I. C. 1 exhibits a high degree of self-incompatibility, although it has abundant pollen and seeds fairly well with pollen of *Musa malaccensis*. Fifty-seven inflorescences produced twelve seeds of which only two germinated, when pollinated by hand. Left to natural pollination 119 bunches produced but nineteen seeds, of which only three germinated. The vegetative habit of all of these is similar to I. C. 1. In three cases, however, the somatic chromosomes were 44, while in the other two they were 46. A giant cell in mitosis gave 92 chromosomes.

Back-crosses on Gros Michel

Pollen of I. C. 1 used on the female parent, Gros Michel, set even less seed than I. C. 1 itself. The relative compatibilities of the original parents

and the hybrid were as shown below, when pollinations were tried under similar conditions:

Cross	Inflorescences	Seeds set	Germination
Gros Michel × *M. malaccensis* .	35	35	10
Gros Michel × I.C.1 . . .	24	1	0
I. C. 1 × I.C.1	57	12	1

The back-cross seedling was very slow in growth and attained a height of six feet only at two years of age, when it flowered. The bunch and fruits exhibited characters of the male parent, though the fruits were parthenocarpically developed and seedless. The male flowers were few, irregular and quite sterile.

The pollen mother-cells from the terminal (male) bud, were remarkably irregular in shape, varying from oval to abnormally elongated. Occasionally two or more cells are closely joined, though at a late stage of development. The nucleus is often at one end or on one side. The anaphase figures are most abundant and chromosomes straggle very loosely on the spindle. Chromosome counts gave 37 and 42, with 39 as the likely truth. At the first division one cell wall is usual, and highly irregular multicellular "tetrads" result in the second division as in Gros Michel. This plant was evidently an extremely unbalanced form.

Back-crosses on the male parent, M. malaccensis

Pollinations made on the male parent with pollen from I. C. 1 and a sister hybrid, GM×B61, set seeds which germinated freely. When these seedlings fruited, the two families resembled one another very closely. The second family, therefore, considered unnecessary and hence destroyed. Reciprocal matings between I. C. 1 and *M. malaccensis* produced no difference in the progeny.

The whole progeny proved heterogeneous, though varying from one another in minor details only. In vegetative habits, they mostly resembled Gros Michel, but the fruit bunch was intermediate between the two parents in its size and position. All were parthenocarpic and set seeds occasionally in some cases. The shape of the fruit and of the terminal bud varied most. Segregation on any simple numerical basis was not observed.

The somatic chromosome complements numbered 33 in fourteen individuals. In three cases, they were 34, showing that much of the viable pollen of I. C. 1 carried 22.

In five individuals studied, the meiotic divisions were seen to be similar and in one of them more closely so. In the first metaphase, a compact plate is formed and as the chromosomes separate, multivalent associations are seen. There is little lagging in the anaphase. At this stage, the chromosome counts are short of expectation, and on searching, the cytoplasm reveals obliterated chromosomes. At the close of the irregular first division, wedge-shaped cells are seen cut off between the main daughter cells in the dyad.

Although the second division begins with nuclei of varying constitution, it is fairly regularly equational. Polar views of metaphase give counts from 13 to 20, with 16, 15, and 14 as the commonest. The tendency of both divisions, therefore, seems to be towards the elimination of 11 chromosomes, and formation of 11-chromosome pollen grains. The process is not seen to be complete.

Discussion

It is thus evident that the chromosome number in the "normal" F_1 hybrids and the increased pollen fertility presumably resulting from it support the view that the basic haploid chromosome number in bananas is 11. Crossing of *M. malaccensis* with Gros Michel as well as with some other edible bananas results in a combination of the female somatic number with the male haploid number. A case of doubling of the male component together with pollen fertility is also mentioned by White. The explanation of this phenomenon, however, is not clear.

The 44-chromosome hybrids obtained from Gros Michel and *Musa malaccensis* differ physiologically in their resistance to Panama disease, although they look like identical genotypes in other respects. Several ways seem to present themselves in which this 44-chromosome complement is possibly made up, by descent from two parents having 33 and 22 somatic chromosomes, respectively. A similar case of the possibility of chromosome increase after reduction is cited in sugarcanes by Bremer, which supports the present hypothesis that the female chromosome complement passes on undiminished, while only a haploid complement is transferred by the male parent into the hybrid progeny.

About twenty per cent of the hybrid seedlings exhibit a higher chromosome number than the sum of the somatic numbers of their parents, and their explanation is difficult to guess.

The hybrids, like the I. C. 1, show that they receive their main contribution from the female parent, but the constitution of this parent itself is not known. The parent, Gros Michel, however, seems to be a triploid and a descendent of the *Musa malaccensis* group. The added complication of secondary polyploidy is also suggested in the case of bananas. However, the possibility of raising a tetraploid from a commercial triploid, and restoring it back to the triploid condition is demonstrated in these investigations and the practical results obtained are very satisfactory.

2. Hybrids of the Mysore banana [Cheesman, 1932]

An edible type named "Mysore" (Yella Bale) imported into Trinidad from Southern India (and perhaps named after the Indian State, Mysore) was also crossed by pollens from *Musa malaccensis* by Prof. Cheesman "in quest of genetic information". The Mysore is described by him as identical with another type called locally Fillbasket. This variety is said to be immune to the Panama disease, tall and vigorous. Leaves are dark green with reddish flush on midribs and on the lower surface of young leaves. Fruits are small, round, and the bunch is compact. Fruit is about seven inches long, with a pronounced beak, thin skin, and bright golden yellow colour when ripe. Somatic chromosomes number 33.

Twenty-seven inflorescences of Mysore pollinated by *M. malaccensis* yielded 198 seeds in 1929-30, single bunches of fruits yielding as many as fifty-one and thirty seeds each, averaging at seven seeds per bunch. But the seeds germinated poorly. Six of the fifteen seedlings obtained, died in the pot and nursery stages. The study recorded here is therefore of the remaining nine seedlings only with notes on the Mysore × I. C. 1 cross.

In the Mysore meiosis in the pollen mother-cell is the same as in Gros Michel. A compact plate is formed at metaphase which has multivalent associations, not analysed. Some chromosomes lag at anaphase, and stragglers excluded from the daughter nuclei are very prominent in the dyad stage. The second division is semi-regular, with some lagging again. Polar views of metaphase give varying counts both below and above sixteen. Multicellular "tetrads" result, and the mature pollens are very sparse. The number of viable pollens in Mysore variety is very negligible.

F_1 generation

Six of the nine plants mentioned above had 44 chromosomes, and resembled their female parent in vegetative habit, while two had 75 and 73 chromosomes, with thick leaves and dwarf growth. One with 33 chromosomes exhibited wild type habit. The fruits in one plant with normal habit failed to swell without pollination, and developed seeds when they did. Two plants showed parthenocarpy. The thick-leaved plants were similar in habit and chromosome number to the progeny of Gros Michel, and two of these which died prematurely also showed the same habit. The plant with 33 chromosomes was intermediate between the two parents.

In meiosis the usual compact metaphase plate occurs and the semi-regular anaphase exhibits varying number of lagging chromosomes. At the dyad stage isolated chromosomes are seen either cut off by the cell walls or lying free in the main daughter cells. The tetrads are mostly multicellular and the pollen irregular in size, but fairly abundant in quantity.

The cross Mysore × I. C. 1

With considerable difficulty the Mysore (a triploid) was successfully crossed with I. C. 1 (tetraploid), and a tall and dwarf plants were the progeny, with 54 or 55 and about 90 chromosomes each, respectively. The larger plant which frequently gave counts of 55 chromosomes, appears to be a pentaploid, while the dwarf plant which gave clear counts of 90, suggests the approximation to 88.

Section III

Chromosome numbers in the Musaceæ

With a view to test the preliminary conclusion that the basic haploid number of chromosomes in the edible bananas is 11, Professor Cheesman, in collaboration with Mr. L. N. H. Larter of the Jamaica Department of Agriculture, carried out chromosome counts in several varieties of bananas. The work was done at Trinidad, the quarantine at Kew, and in Jamaica, and has thrown considerable light incidentally on the proper classification of the genera and species of this sub-order also. The following is a summary of their work in this line.

Of the three sub-groups into which Baker [1893] divided the genus *Musa* L., namely *Physocaulis*, *Rhodochlamys*, and *Eumusa*, the last named group is alone concerned with the origin of the cultivated bananas. Counts of chromosomes detailed here are, therefore, restricted to this group. However, material available on related sections is also examined and included in this study.

All counts were made on root-tips. At Jamaica and Merton, the material was fixed in a modification of La Cour's 2BE, consisting of—

Chromic acid one per cent	90 c.c.
Osmic acid 2 per cent	15 c.c.
Potassium dichromate ten per cent	10 c.c.
Saponin	0·05 grm.
Acetic acid five per cent	20 c.c.
Distilled water	25 c.c.

In subsequent treatment the material was cleared in chloroform, embedded in sixty per cent wax, cut at 20 μ and stained with Newton's Gentain violet.

Chromosome numbers in fertile species of Eumusa

Musa accuminata Colla includes several forms such as *M. simiarum* Kurz., *M. zebrina* Van Houtte, and *M. malaccensis* Ridley. It is common in the Malaya Peninsula and Archipelago, and occurs in a variety of forms connected by all transitions. Crosses between some of the eight distinct clones

at the Trinidad collection, were found to be fertile and intermediate in characters. Special attention was given to *M. accuminata*, as it is considered to be the probable parent of some of the dessert bananas.

While recording these counts, considerable difficulty had to be overcome in fixing the nomenclature of the varieties under study, and out of 29 chromosome counts on fertile *Musa* varieties, the somatic diploid number of chromosomes in 27 cases was 22, and in two cases 20. These twenty-seven cases represent at least seventeen distinct varieties, belonging to six different taxonomic species. Two counts, which have 20 chromosomes, were of clones of *M. textilis*. These counts agreed with thos eof D'Angremond for *M. zebrina* and with those of White for *M. accuminata*, but White records the somatic diploid number as 24 for *M. zebrina* and *M. zebrina cerifera* obtained from Malacca. Counts recorded here (which include material from Yella Bale, *M. sapientum*, a Mysore variety) therefore confirm the view that the basic haploid chromosome number is 11 in the *Eumusa* group.

A *Musa textilis* clone differed from other *Eumusa* species in several respects, and would not cross readily with others. A hybrid of *M. textilis* with Yella Bale (*M. sapientum*) from Mysore, however, has been raised, and is under study. It is suspected that some of the distinct varieties found in the Philippines might have originated from the crossing of *M. sapientum* and *M. textilis*.

Some undetermined species are collected at Trinidad Imperial College of Tropical Agriculture, one of which has the habit of edible bananas, but is very fertile with hermaphrodite flowers at the base of the inflorescence. Two of these were obtained from the Royal Botanic Gardens, Calcutta, under the common Burmese name "Taw Byaw".

Chromosome numbers in sterile clones of Eumusa

The great difficulty in studying edible bananas due to their indefinite nomenclature has been fairly overcome by comparing living plants of several varieties, received under different labels and planted side by side. Study of nearly a hundred varieties during the past ten years, in which chromosome counts formed part of the comparison, has enabled the varieties to be reduced to less than thirty, twenty-five of which are considered distinct types. Some of them have sub-types resulting from bud mutations.

In identifying and classifying the many original plants received, the original labels of each are kept intact, and new numbers and standard names are adopted for each on the basis of the groups in which they fall. Each number and standard name denotes a type, and merges several synonyms in it. The name of the country of origin is also maintained in each case, in order to avoid confusion, arising from the same name being used for different varieties in different countries. Difficulty is still noted in the case of a few varieties in determining the true countries of their origin.

From the cytological studies of Professor Cheesman and Mr. Larter, it is clear that in general *Eumusa* species have 22 or 33 chromosomes. In the sterile varieties the chromosomes are 33 except in four varieties studied, where they are 22 only. Edible varieties are, therefore, generally triploid. These studies bring out a fact which materially differs from White's findings, based on 32 and 36 chromosome counts recorded by him. The present view is in agreement with D'Angremond's results, and is supported by a great deal of evidence. Individual discrepancies noticed in counts of chromosomes are attributable to fragmentation, as observed in Ventricosa, Branca, and several clones of Cavendish. In Branca 32 chromosomes *plus* a fragment are seen, which may be chimerical. In Cavendish three conditions of fragmentation are noticed, namely, 33 chromosomes, 32 chromosomes and two fragments, and 33 chromosomes and one fragment (the fragment possibly being a detached portion of one of the normal chromosomes). Fragments are noticed in Canary and other clones also, and fragmentation is perhaps connected with the exceptionally high degree of sterility found in breeding from them.

Occasionally a doubling of chromosomes, as well as binucleate cells and cells with three or more nuclei also, are seen. As previously pointed out, tetraploidy in the hybrids from Gros Michel is not satisfactorily explained by the hypothesis that unreduced eggs might be functioning. The more satisfactory explanation of tetraploidy, based on the present observations, is perhaps that somatic doubling of chromosomes occurs in the zygote after fusion of the haploid gametes. It is also possible in the alternative, that the fusion or loss of chromosomes occurs during mitosis, because different roots of the same Jamaica seedling gave counts of 31, 32, 33, and 34, chromosomes.

Chromosome numbers in other sections of Musa

One species of *Physocaulis* examined, namely, *M. ensete* gave the somatic diploid chromosome number as 18. White [1928] also found the same number in *M. Gilletti*. This observation suggests the possibility that in Physocaulis, the basic haploid number of chromosomes is 9.

Three varieties of sub-genus Rhodochlamys, namely, *M. coccinea*, *M. violascens*, and Musa species undetermined from Sarawak, have given counts of 20 chromosomes each. The two counts of 20 chromosomes in clones of *M. textilis* recorded above under Eumusa, and the differing vegetative habit of one of them may be now interpreted to suggest that these two clones are more closely related to Rhodochlamys than to Eumusa. Similarly, cytological evidence shows that *M. ornata* would rightly fall under Eumusa rather than under Rhodochlamys. Some other species also show greater affinity for *M. ornata* than for *M. violascens*. This suggests that Baker's grouping under Rhodochlamys is not quite natural. These studies show that the probable basic haploid number in Rhodochlamys is 10.

Chromosome numbers in other Musaceæ

Owing to the varying number of basic haploid chromosomes in the different sub-genera of Musa as noted above, counts of other species of *Musaceæ*, namely *Heliconia*, *Strelitzia*, and *Ravenala*, were also made with a view to ascertain the true and common basic haploid number for the whole sub-order, if possible. This end, however, has not been reached.

Six varieties of *Heliconia* were studied. They indicated a diploid condition. One of them had 22 and the rest 24 chromosomes in them, the pairs varying in their lengths. One of the 11 pairs seen in the first variety was very long, perhaps due to the fusion of two normal pairs together, or it may be that fragmentation occurs in the remaining five varieties, which fact accounts for the larger number of chromosomes found in them.

By similarity of chromosomes, *Strelitzia* and *Revenala* appear to be more closely related to one another than to *Haliconia*. The chromosome morphology of these two genera also is similar to that of *M. coccinea* and *M. textilis*. *Ravenala madagascariensis* has a diploid chromosome complement of 22. *Strelitzia Reginae* has a complement of 14 chromosomes, and stands alone with a number lower than 11.

These cytological findings, therefore, confirm the conclusion drawn by Humphrey that *Musaceæ* are a heterogeneous group. The observations recorded here emphasise the heterogeneity not only in the family *Musaceæ* but within the genus Musa itself.

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ABSTRACT

Fibre-maturity of Indian cottons. A. N. GULATI and N. AHMAD (*I. C. C. C. Tech. Bull., Ser. B, No.* **20**).

Among the physical properties of cotton fibre the mean fibre-length and fibre-weight per inch have been regarded important for some time past. Recent work has brought out the importonce of another property namely, fibre-maturity, which is a measure of the thickness of the cell wall of the fibre in relation to its ribbon width. This property is determined by grading fibres under a microscope after they are swollen with 18 per cent caustic soda solution. The influence of environment and heredity on this property and its relationships with other fibre properties and the yarn characteristics of cotton formed the subject of an investigation, which was recently undertaken at the Technological Laboratory and described in the Technological Bulletin, Series B, No. 20.

For the purpose of this investigation thirty-two cottons were selected, out of which thirty were grown in India. The results obtained were analysed with the object of studying (1) the effect of season, locality of growth and heredity on fibre-maturity, (2) the relationships between the fibre-maturity and other principal fibre properties and (3) the influence of fibre-maturity on the neppiness and strength of the yarns spun from these cottons.

It was found that in some cottons fibre-maturity fluctuated from season to season, while in others seasonal fluctuations were quite small. Gadag 1 and Karunganni C.7 are examples of the former while C.A.9 and Mollisoni illustrate the latter. It was further found that samples of Banilla cotton grown in different parts of Khandesh showed significant differences as regards fibre-maturity, while further selections from P.A. 289F grown at the same place (Lyallpur) also showed significant differences. These results indicate the desirability of exercising caution in supposing that selections will follow their parental stock in respect of fibe-maturity or that a cotton which gives good results in respect of fibre-maturity when grown at one place will behave equally well when grown at another place even in the same cotton growing tract.

As regards the relationship between fibre-maturity and other fibre properties, it was observed that on the whole the short-stable cottons possess high fibre-maturity, while the long-staple cottons generally possess a relatively higher percentage of immature fibres. Fibre-weight and fibre-strength were found to be positively correlated with the percentage of mature hairs in a cotton.

The most important effect of the presence of immature hairs in a cotton is the production of neps in the yarns spun from it. The half mature hairs also appeared to be a secondary cause in the formation of neps. The correlation coefficient between the combined percentage of immature and half mature hairs on the one hand and the number of neps per yard of yarn on the other was found to be as high as +0·67 for twenty-ieght samples. These experiments

definitely show that cottons possessing a high percentage of immature and half mature hairs should, so far as possible be avoided, as they would yield neppy yarns.

As regards the effect of fibre-maturity on yarn strength, it was found that in long and medium staple cottons, high fibre-maturity went hand in hand with high yarn strength, while in the short staple cottons the reverse was the case. Thus, in the selection of medium and long staple cottons, due regard should be given to fibre-moturity besides the other fibre properties like mean fibre-length and fibre-wight per inch.

Full details of the investigation will be found in the bulletin referred to above (*Authors' abstract*).

NOTE

RESOLUTIONS CARRIED BY THE SIXTH INTERNATIONAL BOTANICAL CONGRESS (AMSTERDAM, SEPTEMBER 2—7, 1935)

1. General Resolution

It is proposed that the Botanical Section of the International Union of Biological Sciences should act as an administrative connecting link between the successive International Potanical Congresses, which maintain their full independence from an international point of view to such an extent that any country which has not joined the Union, will have quite the same rights as those which have joined.

It is proposed that this Botanical Section be authorized to carry through any resolutions, carried by the International Botanical Congresses.

Proposed by A. C. SEWARD and seconded by J. C. SCHONTE, C. J. BUTLER, E. D. EERRILL, N NEMEC, M. J. SIRKS AND N. E. SVEDELIUS.

2. General Resolution

By reason of the growing financial difficulties and the prohibitive price for certain museums and laboratories, of transmission by post, rail and boat, it becomes impossible to guarantee the indispensible exchange of collections of dried plants. It is therefore necessary to obtain for this exchange the free transport now accorded to the exchange of scientific publications.

The Congress asks the Office of the International Union of Biological Sciences to take the necessary steps with various governments in order to obtain this postal privilege.

Proposed by B. G. P. HOCHREUTINER and seconded by H. HUMBERT, E. D. MERRILL, A. A. PULLE and C. SKOTTSBERG in the name of the meeting of Directors of Botanical Gardens.

3. Section Agr.

That the Seventh International Botanical Congress shall arrange a Section for Agronomy on the same lines as that of the Sixth Congress.

Proposed by the Section for Agronomy.

4. Sections Cyt. and Gen.

That the Botanical Section of the International Union of Biological Sciences on behalf of the Sixth International Botanical Congress may apply to the International Committee for Genetical Congresses for the appointment of an International Committee to study the different meanings of various cytological and genetical terms used in the literature, to propose exact definitions of these terms and to suggest if and what further steps are necessary to improve the terminology of genetics and cytology.

Proposed by F. W. SANSOME and seconded by M. J. SIRKS.

5. Sections Cyt. and Gen.

The Sections of Genetics and Cytology of the Sixth International Botanical Congress take the occasion of their presence in Holland to record the deep sense of debt which the sciences of Genetics and Cytology owe to Hugo de Vries. It is a satisfaction that he could live to such a ripe old age and thus see imposing structure which has been erected on the foundations in the laying of which his painstaking investigations and prophetic vision had so large a share.

The Honorary Secretary of the Congress is hereby requested to spread this resolution on the minutes of the Proceedings and to send a copy to Mrs. de Vries with the sympathy of the Congress and with the assurance that Hugo de Vries will remain for all time an inspiration to biologists and live as one of the immortal heroes of Science.

Proposed by A. F. BLAKESLEE and seconded by F. OEHLKERS and E. W. SINNOTT.

6. Section Geo.

The Sixth International Botanical Congress at Amsterdam, 1935 appeals to all botanists and cartographists with the request that they should employ for the same plant communities the same colouring and for sociologically related plant communities, especially in survey maps, similar tints, in accordance with the proposals which the Fifth International Botanical Congress put forward at Cambridge. Should the case arise necessary modifications shall be accounted for.

7. Section Geo.

The Sixth International Botanical Congress draws the special attention of Public Bodies, Scientific Institutes and Societies for the International Protection of Nature to the regrettable danger of inconsiderate destruction of primitive vegetation to which, in the future vast territories especially in tropical and sub-tropical countries, will be exposed through the nefarious practice of brushwood and prairie fires.

It points out the often radical changes brought about by this practice in the biologic complex constituted by the community of plants and animals. On the one hand, a very great number of species are threatened in a short time with total extinction; many of them being of the greatest interest from the scientific, aesthetic and economic points of view. On the other hand, countries formerly covered with a woody vegetation of various types, are becoming, or are in process of becoming, deserts in consequence of the changes which the destruction of this vegetation produces upon the local climate, the hydrologic conditions and the physico-chemical characters of the soil.

The Congress emprasizes the necessity:

(1) Of constituting in these countries permanent and fairly extensive reserves for safeguarding the equilibrium of the natural conditions and the living members of the vegetable and animal kingdoms (National Parks);

(2) Beside the territories thus constituted as permanent Reserves, to lessen in some measure the consequences of the deforestation and exploitation by maintaining an adequate proportion of forest areas, taking this in its widest sense;

(3) To prevent the substitution of natural formations of woody autochtones by those of exotic species (in accordance with the conclusions of the International Conference for the Protection of the Fauna and Flora of Africa, London 1933).

Proposed by H. HUMBERT, P. G. van TIENHOVEN and seconded by R. BOUILLENNE, J. BURTT DAVY, A. CHEVALIER, L. EMBERGER, A. W. HILL, J. JESWIET, P. LEDOUX, R. MAIRE, E. D. MERRILL, G. NEGRI, I. B. POLE EVANS, J. RAMSBOTTOM and A. WILCZEK.

8. Section Geo.

The Sixth International Botanical Congress is conscious of the great dangers that result from the destructions of tropical and subtropical vegetations, and also of the perturbations caused in such regions by the action of man.

The Congress insists upon the study of these problems by the botanists of these countries and that they may inform their governments.

The Congress demands especially that these informations shall be collected and that they shall be published in view of a most efficient protection of the indigenous vegetation.

The Congress appoints a Committee for studying this subject and for submitting a report to the Seventh Congress. The following members of this Committee are nominated: I. B. POLE EVANS, president; H. HUMBERT, E. D. MERRILL and J. NABELEK, vice-presidents; R. BOUILLENNE, H. BROCKMANN

JEROSCH, A. CHEVALIER, L. EMBERGER, A. W. HILL, J. JESWIET, P. LEDOUX, R. MAIRE, G. NEGRI, L. R. PARODI and M. F. GALLEGO QUERO and as Secretary J. TROCHAIN.

Proposed by I. B. POLE EVANS and seconded by H. HUMBERT.

9. Section Geo.

That the programme of the Seventh International Botanical Congress shall contain the study and the delineation of the characters of the various types of steppes, their origin, their development and especially the discriminative criteria for climatic associations and secondary associations which get confused under the names "steppe", "prairie", "Savannah" and the definitions of the corresponding phytogeographic regions.

Proposed by H. HUMBERT and seconded by L. EMBERGER, H. GAMS, R. MAIRE, R. BOUILLENNE, A. EIG, G. NEGRI, J. TROCHAIN, H. BOJKO, P. LEDOUX, A. CONARD, E. WILCZEK, E. SCHEID and J. PODPERA.

10. Section Geo.

A committee shall be appointed to elaborate proposals for a classification of climates from a phytogeographical point of view and to submit them to the Seventh International Botanical Congress.

Proposed by L. EMBERGER, and seconded by H. BROCKMANN JEROSCH, S. P. AGHARKAR, A. CHEVALIER, A. EIG. H. GAMS, H. HUMBERT, P. LEDOUR, T. LIPPMAA, R. MAIRE, G. NEGRI, R. PAMPANINI, I. B. POLE EVANS, J. TROCHAIN.

11. Section Geo.

The Geobotanical Section of the Sixth International Botanical Congress recommends:

(1) to use the term *Sociation* for vegetation units characterized mainly by dominance in the different layers, in the sense of Scandinavian plant sociologists;

(2) to use the term *Association* for vegetation units characterized mainly by characteristic and differential species in the sense of Zürich Montpellier plant sociologists, or at least for units of the same order of sociological value; *Subassociation* and *Facies* can, where necessary, be used for their subordinate units;

(3) to unite sociations and associations into alliances in the sense of Zürich-Montpellier plant sociologists, and the alliances into higher units.

Proposed by J. BRAUN BLANQUET, G. E. DU RIETZ and R. NORDHAGEN.

12. Sections Myc. and Path.

The Sixth International Botanical Congress wishes to express its sincere appreciation of the admirable work accomplished by the Centraalbureau voor Schimmelcultures at Baarn since its foundation in 1906 and views with grave concern the present financial difficulties of what is essentially an international institution.

Proposed to the Section Myc. by J. Ramsbottom and seconded by E. J. Butler; to the Section Path. proposed by E. E. Rhiem and seconded by E. C. Stakkan.

13. Section Path.

The Committee on Description and Nomenclature of Plant Viruses appointed by the Fifth International Botanical Congress, 1930, wishes to report that it has made progress in developing a scheme for the nomenclature of plant viruses and suggests to this congress that the Committee be empowered to continue its considerations and establish an acceptable system of virus nomenclature.

Proposed by H. M. Qaunjer and seconded by James Johnson, P. A. Murphy, J. Henderson Smith and G. Samuel.

14. Section Path.

It is recommended that the term "physiologic race" be substituted for "physiologic form" as the former seems more appropriate. It is recommended further that the word "race" be used in general to designate biotypes or groups of biotypes that differ from each other in physiologic characters.

Proposed by E. C. Stakman and seconded by Miss M. Newton, Miss G. Millbrink and Th. Roemer.

15. Section Path.

The Sixth International Botanical Congress expresses itself of the opinion:

that an effective and unceasing campaign against destructive plant diseases and insect pests can be successfully persecuted only by international action and mutual co-operation;

that close and frequent international discussion of the problems of plant quarantines should take place to bring about improvement of the health conditions of plants and plant products offered for export;

that it unanimously recognizes that such section will greatly facilitate international trade in the commodities concerned; and

that, finally, this resolution be brought to the attention of the League of Nations, emphatically endorsing the League's proposal to give this matter the urgent and careful consideration with a view to facilitating and expediting the purpose and aims of this resolution.

Proposed by H. T. Gussow and seconded by N. van Poeteren.

16. Section Sys.

The Sixth International Botanical Congress agrees to the following propositions by the International Standing Committee for Urgent Taxonomic Needs:

(1) Compilation of a new Phytography. The book should not treat the whole content of De CANDOLE's "Phytographie"; it should prepare a new compilation of the collections represented in the larger herbaria of the world. The institutes are asked to draw up a list of their collections.

(2) Photographs of Type Specimens. The Scheme to photograph the types of all plants is important, and should be encouraged, but involves very great expenses. As the means of most Institutes are restricted at the present time, this point should be postpond to the next Congress.

(3) International Dictionary of Botanical Terminology. The Dictionary should be restricted to Phanerogams. The Cryptogams are to be worked out by specialists of the individual groups. The Latin word should be translated and explained in English, French and German. For use in countries of other languages the botanists of such countries should supply translations. All terms would be given in alphabetical sequence. At the end, the most important terms for single categories (f. i. leaf, root, Orchids) might be placed together once more.

(4) It is highly desirable, that the specimens of the Linnean type specimens at the Linnean Society and at the British Museum should be photographed.

Proposed for the International Standing Committee for Urgent Taxonomic Needs by L. DIELS and seconded by A. D. COTTON, R. FRIES, R. MAIRE, E. D. MERRILL and J. RAMSBOTTOM.

17. Section Sys. Subsection Nom nclature

Votes of Thanks.—To the Editorial Committee of the International Rules of Botanical Nomenclature, ed. III (1935), namely Professor HARMS (General Editor), Dr. RENDLE and Professor HOCHREUTINER, for the successful manner in which they carried out their difficult task.

To the Executive Committee of the Fifth International Botanical Congress, Cambridge, 1930, for defraying the cost of publication of the "Synopsis of Proposals" and "Preliminary opinions" concerning nomenclature.

Resolution.—That the Sixth International Botanical Congress accepts the decisions of the Section of Taxonomy and Nomenclature concerning the modification of the International Rules of Botanical Nomenclature, ed. III (1935) and sanctions the appointment of the Standing Committees by this Section.

Proposed by E. D. MERRILL and seconded by L. DIELS.

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ORIGINAL ARTICLES

ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY

PART IV. INTERPRETATION OF TITRATION CURVES

BY

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(With twenty text-figures)

The work on the titration curves of silicic acid sols [Mukherjee, Roychoudhury, Das Gupta and Chatterjee, 1932; Mukherjee, Roychoudhury, Mukherjee and Majumdar, 1934] has been extended. The method of preparaing the sols was as previously reported [Mukherjee *et al.*, 1932] and as small amounts of hydrochloric acid and sodium chloride are not completely removed by the dialysis, several sols and their ultrafiltrates have been analysed for this purpose. The peculiarities of the titration curves observed previously required further investigation. The curves were often not exactly reproducible and sometimes showed [compare curves I, II, IV, V, VI, for sols I, II, and III; Mukherjee *et al.*, 1932] changes in curvature simulating the steps in the curves of a polybasic acid representing particular stages of its dissociation. Irregularities have also been observed [Sol A and its dilutions; Mukherjee *et al.*, 1934] with sols having visible aggregates suspended in them. In the present paper the titration curves of a series of silicic acid sols obtained with considerably improved experimental arrangements have been given. Different concentrations of alkalis and different alkalis have been compared. The course of interaction between the sol and neutral salts has been followed. The hydrogen electrode gives troubles in extremely dilute unbuffered acid systems and the extent and manner of variations of the e.m.f. have been noted. The pH values obtained from the hydrogen electrode have been compared with corresponding values from the quinhydrone electrode.

Experimental

The sols were prepared and purified as previously reported. The accidental ingress of carbon dioxide, oxygen and impurities from the atmosphere both into

the stock sol and the titration cell during a titration forms an important source of error. The apparatus for stocking the sol in an atmosphere of hydrogen gas has been modified by using mercury seal stop-cocks to the exclusion of all rubber connections. The new arrangement (Fig. 1) was found to maintain a vacuum of 750 mm. of mercury for days. The titration cell for simultaneous potentiometric and conductometric measurements [Mukherjee, *et al.*, 1932] has also been modified by the introduction of ground-in mercury seal stirrers (Fig. 2) in order to secure a rapid and efficient intermixture between the sol and the titrant liquid. In view of the high resistances of the systems precautions had to be taken against electrical leakages. The working table and wall plugs were suitably guarded with insulated, earthed metallic shields (copper, except for the table which was covered with galvanised sheets) and similar arrangements were used for the cells and the electrical heating assembly used for the thermostat. All exposed terminals and connecting wires were also similarly protected. These modifications in the experimental arrangement gave e.m.f.'s constant to within 0·1 millivolt and also worked satisfactorily in other regards. A Leeds and Northrup K-type potentiometer and a galvanometer with a current sensitivity of 10^{-11} ampere were used. All measurements were carried out in an electrically heated water thermostat maintained at a temperature 35°C.$\pm$0·05.

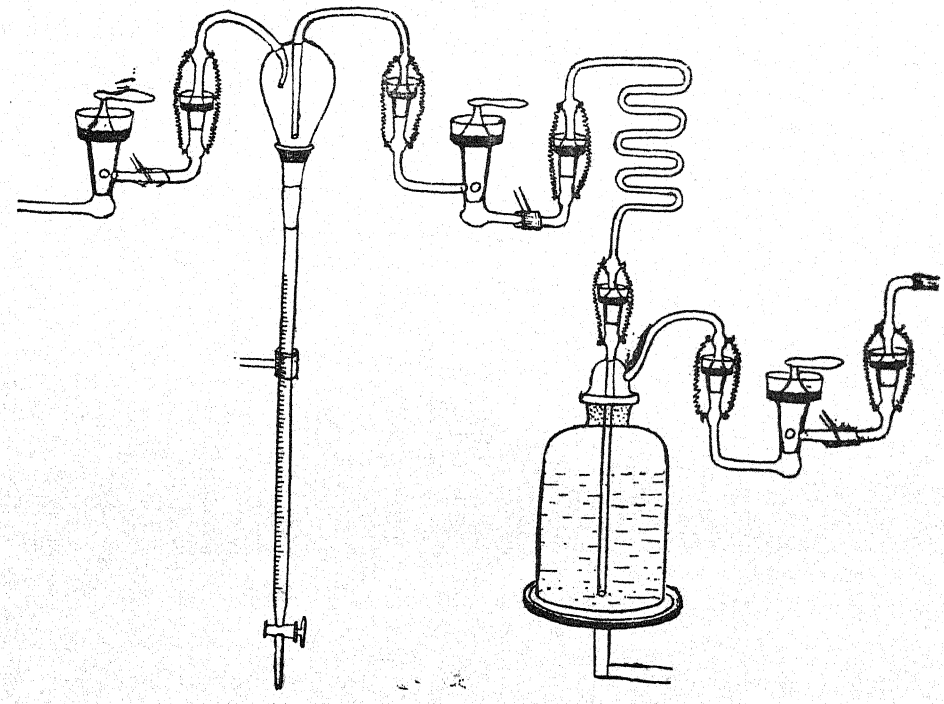


Fig. 1. Stocking arrangement.

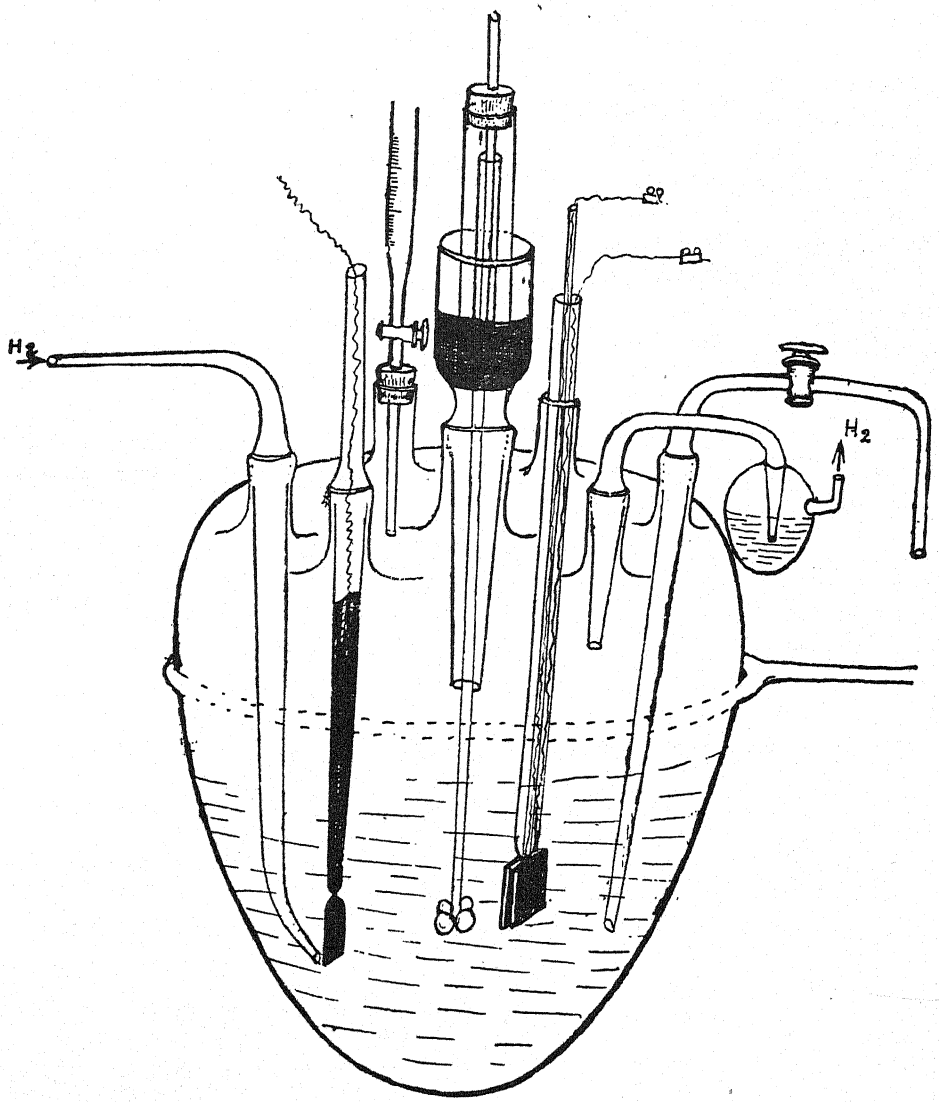


Fig. 2. Titration cell.

Reproducibility of results

The slopes of the titration curves afford useful information and the reproducibility of the curves is of particular importance. As already stated the curves given in previous papers were often not exactly reproducible and they sometimes showed more than one change in curvature resembling the steps in the curves of

a polybasic acid. The titration curves obtained with the improved experimental technique are free from the kinks previously observed. There is no evidence of stages of neutralisation characteristic of polybasic acids in any of the curves given in this paper. This is of interest in view of polybasicity attributed to colloidal clay acid by some soil chemists.

The pH of the sols as observed with the hydrogen electrode sometimes shows wide variations. The quinhydrone electrode is not suitable for titration up to alkaline regions and to obtain a continuous series of measurements with the same arrangement it is preferable to use the hydrogen electrode. Possible adsorption of the quinhydrone by the colloidal particles also makes it desirable to compare its results with those of the hydrogen electrode. The pH obtained with the latter has been often checked as in the previous work with the quinhydrone electrode. Although the pH with the hydrogen electrode often differs considerably for the same sol, the titration curves are mostly identical in form and run parallel, indicating that the electrode maintains a constant hydrogen-ion solution tension throughout the titration and a constant condition of the surface. This observation is of considerable interest. Curves 3 and 4 for sol E (Fig. 4) may be cited as an illustration. Curve 4 represents a titration which was continued for four days and was discontinued each evening being taken up next morning. The curves are remarkably smooth considering the extremely small quantities of acids present. Curves 1 and 2 for sol D (Fig. 3) constitute another example.

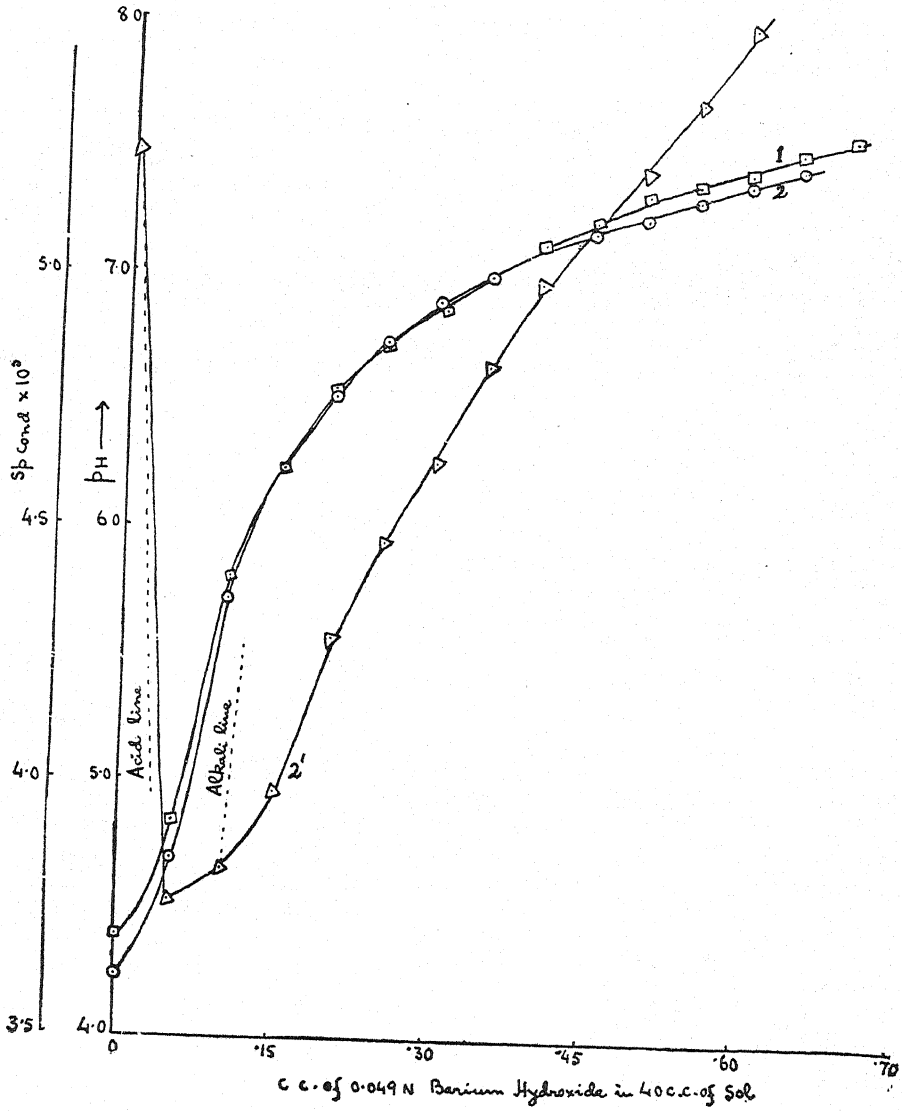


Fig. 3. Silicic acid sol D.

The variations in the pH as also the specific conductivities observed during this work are given in Table I. Q_h denotes results with the quinhydrone electrode; the other values are those of the hydrogen electrode. The observed e.m.f. against the normal calomel electrode is given in the first column; the e.m.f. (E_h) of the experimental cell against the normal hydrogen electrode is given in the second column and in calculating it the e.m.f. of the normal calomel electrode has been taken to be 0·2823 volts at 35°C. The maximum difference from the average E_h is given in percentage of the average value in the third column; the pH calculated from the hydrogen and the quinhydrone electrodes are shown separately in the fourth column. The specific conductivities† are given in the last column.

TABLE I

E_{obs} (in volts)	E_h (in volts)	Maximum difference from average E_h (in percentage)	pH	Sp. conductivity in mhos.
Sol D. 0·5416	0·2593		4·24	
0·5510	0·2687	±1·78	4·39	$5·24 \times 10^{-5}$
	average: 0·2640		average: 4·32	
Sol E. 0·4993	0·2170		3·55	
0·5042	0·2219	—0·92	3·63	$3·76 \times 10^{-4}$
0·5028	0·2205	+1·23	3·60	
	average: 0·2197		average: 3·59	
0·1895(Q_h)	0·2198(Q_h)		3·59(Q_h)	
Sol F. 0·5089	0·2266*		3·70*	
0·5089	0·2266*		3·70*	
0·5480	0·2657		4·35	
0·5447	0·2624	—0·87	4·29	$5·20 \times 10^{-5}$
0·5445	0·2622	+1·18	4·29	$5·43 \times 10^{-5}$
0·5426	0·2603		4·26	
	average: 0·2626		average: 4·29	average: $5·32 \times 10^{-5}$
Sol G. 0·6095	0·3272		5·36	$1·40 \times 10^{-5}$
0·6120	0·3296		5·39	
0·6128	0·3305		5·41	
0·6440	0·3617		5·92	
0·6300	0·3477		5·69	$1·39 \times 10^{-5}$
0·6215	0·3392		5·55	
0·6383	0·3560		5·82	$1·48 \times 10^{-5}$
0·6248	0·3425		5·60	$1·45 \times 10^{-5}$
0·6170	0·3347		5·48	$1·45 \times 10^{-5}$
0·6445	0·3622		5·92	$1·57 \times 10^{-5}$
		—5·56		
	average: 0·3431	+4·63	average: 5·61	average: $1·46 \times 10^{-5}$
Sol H 0·5230	0·2401		3·94	
0·5275	0·2452		4·01	
0·4920	0·2097*		3·43*	
0·5266	0·2443		4·00	$0·891 \times 10^{-5}$
0·5080	0·2257*		3·69	$0·891 \times 10^{-5}$
0·5331	0·2508		4·10	
0·5265	0·2442	—1·0	3·99	
0·5297	0·2474	+2·1	4·05	
	average: 0·2455		average: 4·02	
0·1585(Q_h)	0·2508(Q_h)		4·1 (Q_h)	
Sol H/2. 0·5370	0·2547		4·17	$0·46 \times 10^{-5}$
0·1466(Q_h)	0·2627(Q_h)		4·30(Q_h)	
Sol H/5. 0·1307(Q_h)	0·2786(Q_h)		4·57(Q_h)	$0·21 \times 10^{-5}$
0·1276(Q_h)	0·2817(Q_h)	—0·90	4·61(Q_h)	
0·1264(Q_h)	0·2829(Q_h)	+0·70	4·63(Q_h)	
	average: 0·2811		average: 4·60	
Sol H/10. 0·5352	0·2529		4·14	$0·11 \times 10^{-5}$
0·1240(Q_h)	0·2853(Q_h)		4·67(Q_h)	
Sol H/20. 0·5927	0·3104		5·08	$0·056 \times 10^{-5}$
0·1099(Q_h)	0·2994(Q_h)		4·90(Q_h)	

* These values have been omitted in calculating the average values.

† The variations in the specific conductivities arise from diffusion from the salt bridge.

The limitations of the hydrogen electrode for activity measurements of such dilute unbuffered acid solutions are well known. It has been found that the agreement between the different measurements is often, though not always, improved by cleansing and re-platinising the electrodes. Where a large number of measurements agree between themselves and the average so obtained also gives a pH in agreement with that of the quinhydrone electrode the absolute value of the pH is reliable to the extent of the variations. In other cases we do not lay stress on the absolute value. The unknown small potential drop at the liquid junction of the saturated potassium chloride bridge introduces a minor error. The activity of the hydrogen ions calculated from the e.m.f. is liable to greater error on account of the functional relationship between these two quantities. The extremely small concentration of the acid has also to be taken into account in estimating the strength of the acid indicated by such comparison. The discussion in the sequel is subject to these remarks.

As already mentioned the variations in the intial pH of the titration curves do not materially affect the total acidities calculated from them as the curvature and the course of the curve is mostly independent of them (see, for example, curves 1 and 2, sol D ; 3 and 4, sol E ; 10 and 11, sol F ; particularly curves 18 and 19, sol H). In fact the calculated total acidities have mostly been found to agree within $\pm$ 5 per cent of average values in all cases. The variations are greatest for sol G which is also the sol with the highest pH, the lowest total acid, and which is also the least conducting.

Peculiarities of silicic acid sols

It has been observed [Mukherjee *et al.*, 1934] that the total acid taking part in the reaction with the alkali is indefinite and depends on the peculiarities of surface reaction. Theoretically speaking this need not be the common characteristic of all colloidal acids, or, for the matter of that of different colloidal solutions of the same acid substance. Thus the particles of a finely dispersed sol may react rapidly with the alkali when its concentration is not too low. In such cases the processes responsible for a slow interaction with the hydroxyl ions may be eliminated and the colloidal particles will simulate acid molecules in true solution. Different types of titration curves have been observed [Mukherjee *et al.*, 1934] with dilute sols and with sols which are concentrated or have large aggregated particles. When such concentrated sols are diluted the titration curves conform more and more to the form of moderately strong acids. This change in form may, in part, be ascribed to the same reasons as obtain when a weak acid in true solution is sufficiently diluted and its degree of dissociation becomes large. But indications were also obtained that a slower neutralisation of the hydroxyl ions resembling more an adsorption process than the neutralisation of hydrogen ions also takes place. A comparison of the curves for sol A and its dilutions [Mukherjee *et al.*, 1934] may be cited as an illustration. The conductometric titration curves show

that in the acid region the slope decreases as the neutral point is approached (sols A/6 and A/11). The conductometric titration curve for sol A/2 indicates adsorption near about the neutral point. At higher alkali concentrations another type of slower neutralisation of hydroxyl ions is conceivable and arises out of the dissolution of the colloidal particles.

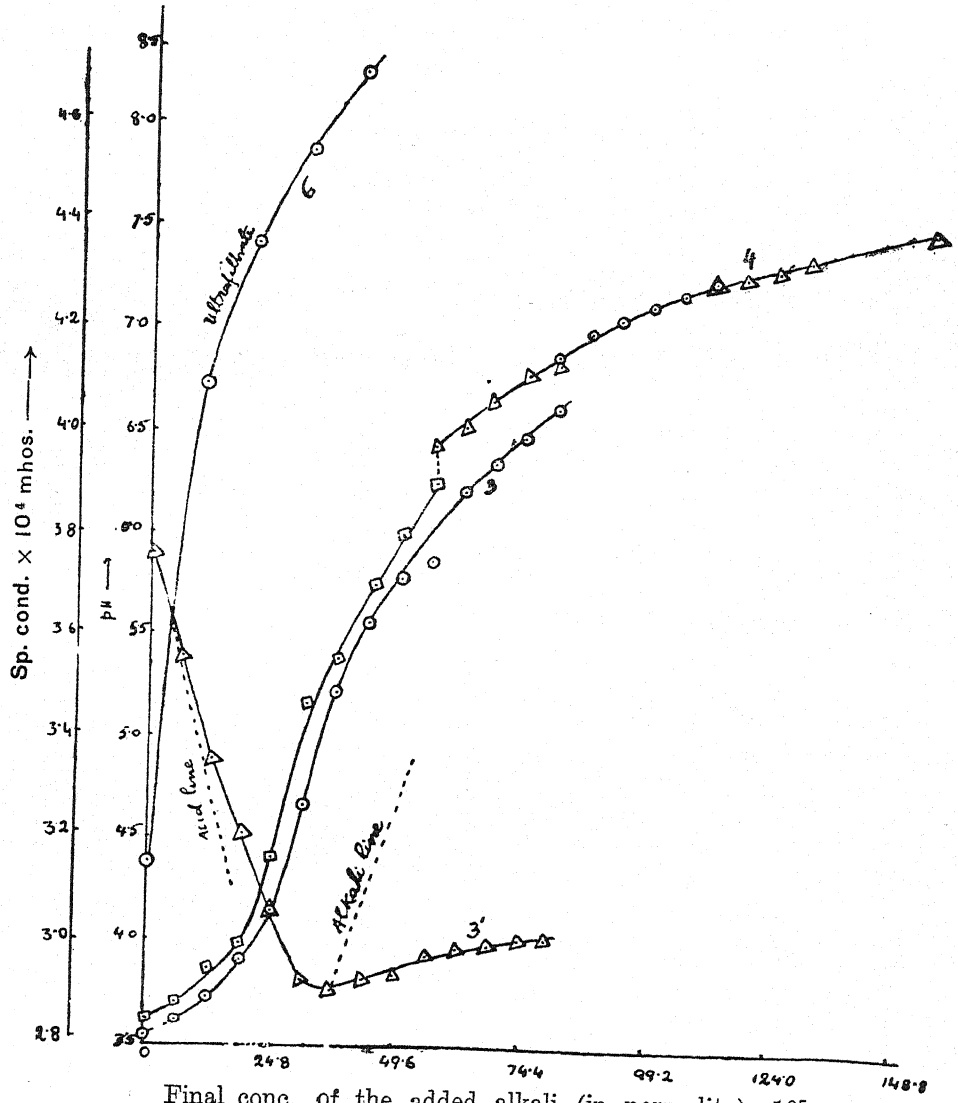
Some of the sols have been found to be more dissociated (Table VI) than others, judging from a comparison of their free and total acidities. The amounts of sodium and hydrogen chlorides contained in them may have some relation to this difference apart from the concentration of the particles. The liberation of acids by salts of alkali and alkaline earth metals of weak and strong acids when they interact with colloidal acids in the sol or gel form is well known and is of general interest, but these interactions do not seem to have been systematically studied.

Sol D

Experiments with this sol were intended to check the efficiency of the experimental arrangement in the way of obtaining reproducible measurements. The potentiometric titration curves 1 and 2, of this sol are remarkably smooth and are almost identical. These curves as also the conductometric titration curve 2′ indicate an acid of a fairly strong character.

Sol E

This sol contained finely-dispersed particles. No aggregates were visible with the naked eye. The ultrafiltrate was obtained from collodion membranes which were previously washed with water of specific conductivity $1 \cdot 34 \times 10^{-6}$ mhos till the wash liquid had a constant specific conductivity of $3 \cdot 0 \times 10_{-6}$ mhos. A nickel plated ultrafilter of Zsigmondy's pattern was used. Traces of nickel were later found to contaminate the ultrafiltrate and the ultrafilter was, therefore, replaced by a porcelain one of the same pattern. 'Cella' membranes of various pore sizes have been used in later work.



Final conc. of the added alkali (in normality) $\times 10^5$.

Fig. 4. Silicic acid sol E.

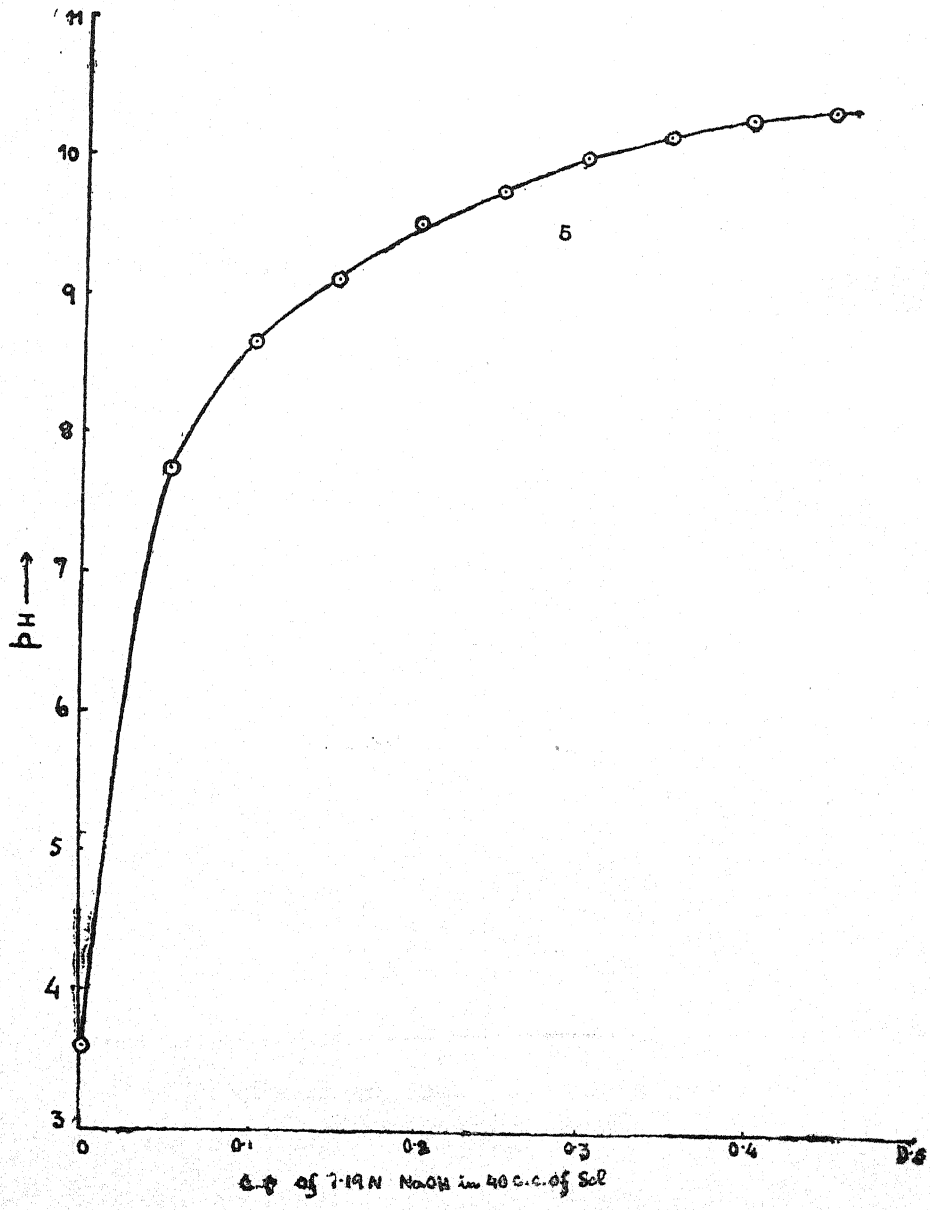


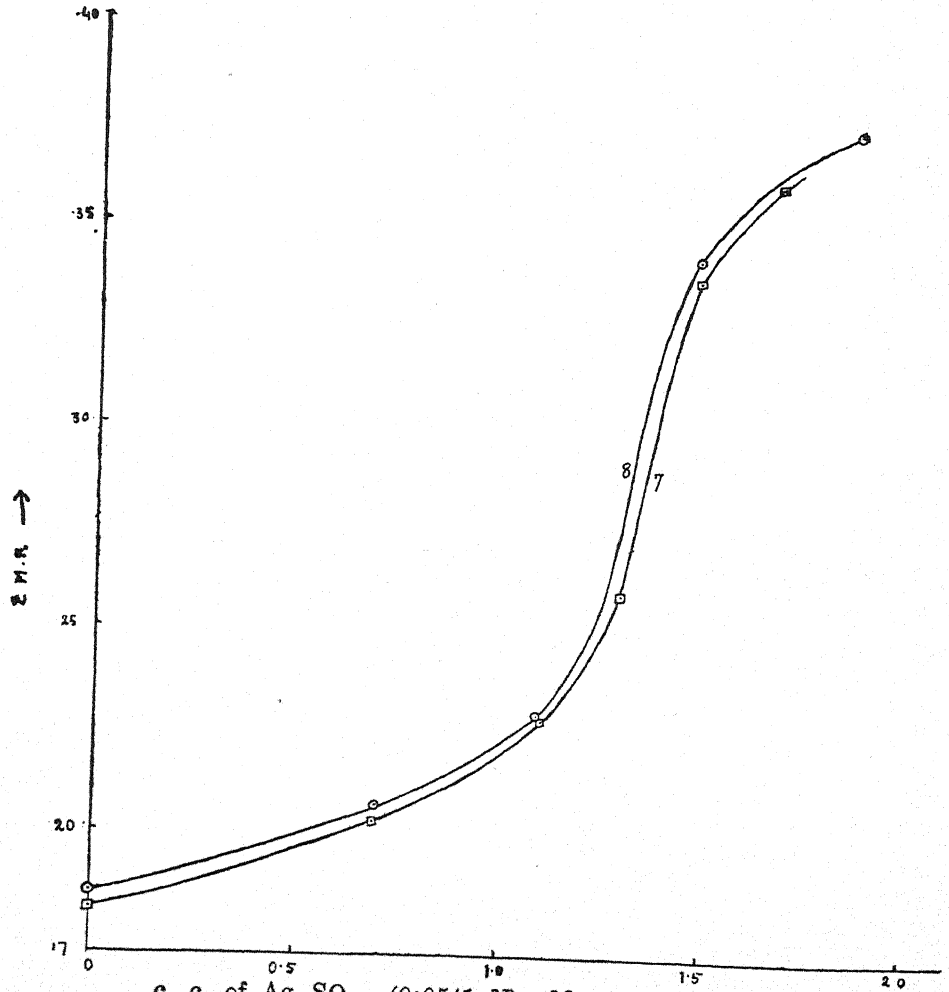
Fig. 5. Silicic acid sol E.

The total chlorine was obtained by both conductometric and electrometric titrations with silver sulphate using Ag-AgCl electrodes in the latter case. The silica and sodium were estimated as follows : A definite volume of the sol after evaporation and ignition of the residue was treated with concentrated sulphuric acid. The residue was then gently heated with additions of drops of concentrated hydrofluoric acid ; the loss in weight (after drying) by this treatment gave the percentage of silica. A blank experiment was performed to ascertain the weight of the non-volatile impurities in the same volume of the hydrofluoric acid. Assuming from the method of preparation that the other non-volatile ingredient of the sol was sodium chloride the amount of sodium was calculated from the weight of silica and sulphate before treatment with hydrofluoric acid. For the preparation of the silicic acid sols Merck's pure sodium silicate was used and as it may contain traces of other substances the amount of sodium estimated as above is liable to some uncertainty. The results with sol E are given in Table II.

TABLE II

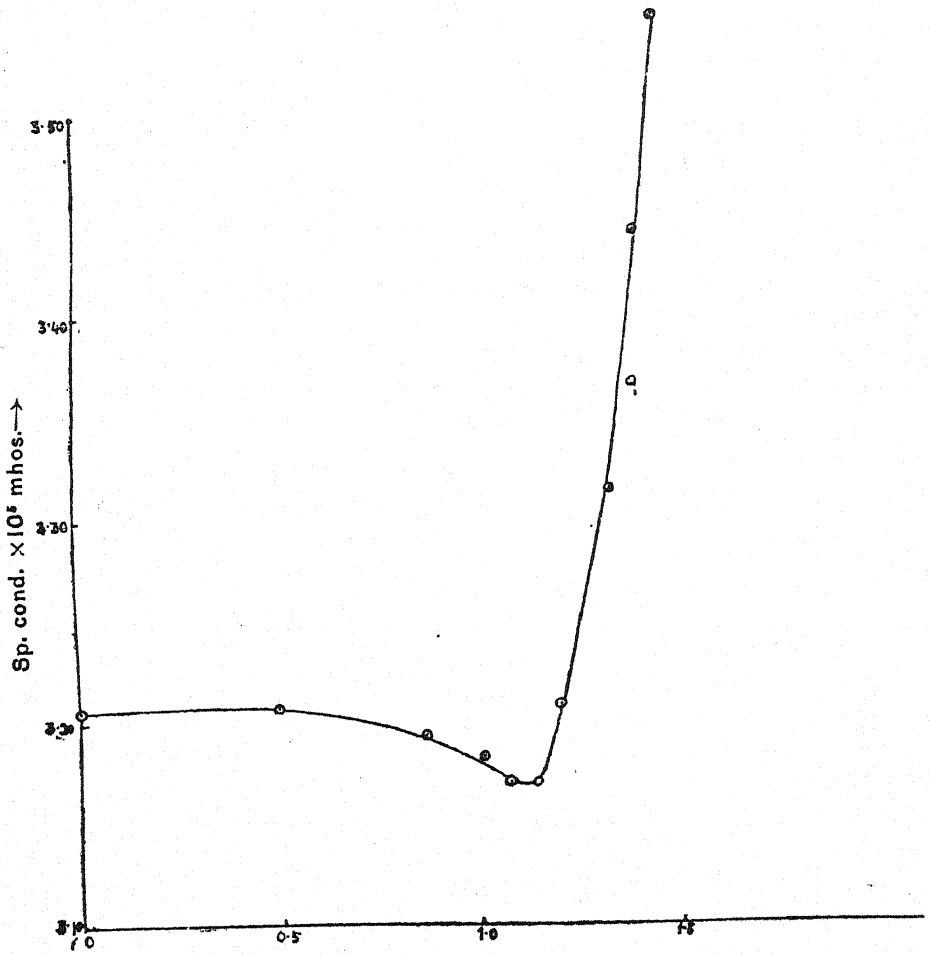
	Gm.-mols. of SiO_2 per litre	Total sodium	Total chlorine	Sp. cond. in mhos $\times 10^5$ (Obs.)	Sp. cond. in mhos $\times 10^5$ (Calc.)	Total acid $\times 10^5$	H-ion conc. $\times 10^5$ (Free acid)	Free acid / Total acid
Ultrafiltrate of sol E	...		0·00153*N* (curve 9) .	2·53			4·17	
Sol E . . .	0·116	0·0014*N**	0·00151*N* (curve 7)					
	0·116	0·00146*N*	0·00151*N* (curve 8) .	3·76	3·44	30·4 (curve 3)		
	0·115	0·00146*N*				30·0 (curve 4)		
	0·115	0·00146*N*	0·00155*N* (conducto-metric)				25·7 (from average pH Table 1)	0·85
	average: 0·1155	average: 0·00146*N*	average: 0·00152*N*			average: 30·2		

Curves 3, 3′ and 4 indicate a moderately strong acid character in agreement with the high ratio of free to total acids. The acidity of the ultrafiltrate (*cf.* curve 6) which may also be taken to measure the concentration of the free hydrochloric acid in the sol is about fifteen per cent of the total acid of the sol. The concentration of chlorine in the sol (curves 7, 8, Fig. 6) agrees well with that in the ultrafiltrate (curve 9, Fig. 7). All the chloride is, therefore, present in the intermicellar liquid and completely passes into the ultrafiltrate. The chlorine is contained mostly as sodium chloride and the remainder as free hydrochloric acid. The conductometric titration curve (curve 9) of the ultrafiltrate with silver sulphate has the usual form of the titration of a chloride by a soluble silver salt. The decrease in the conductivity (about 1·2 per cent) towards the end point is partly due to the dilution of the titrant liquid.



c. c. of Ag_2SO_4. (0·0541 *N*) added to 40 c. c. of sol.

Fig. 6. Sol E.



c. c. of Ag_2SO_4. (0·0541 *N*) added to 40 c. c.

Fig. 7. Ultrafiltrate of Sol E.

The calculated specific conductivity given in column 5 of Table II was obtained as follows: Assuming the sodium and the hydrogen ions to be the only carriers of positive electricity the excess of the sum of their observed concentrations over the concentration of the chlorine ions gives the 'equivalent concentration of the negatively charged silicic acid particles. The observed specific conductivity should be given by the sum of the contributions to the total conductivity due to the individual ion constituents. From the known total concentration of chlorine ions in the sol the concentration calculated per c. c. multiplied by the equivalent conductivity of chlorine ions at infinite dilution at 35°C. gives the contribution of these ions to the specific conductivity. The contributions due to the sodium and the hydrogen ions are similarly obtained, the activity of the hydrogen ions being calculated from the average pH. An equivalent conductivity of 20 has been assumed for the silicic acid particles. The following limiting equivalent conductivities at 35°C. have been assumed for the other ions: $U_{H^\circ} = 404$; $U_{Na^\bullet} = 64$; $V_{Cl} = 93$. It will be found that the observed specific conductivity of the sol is nearly ten per cent higher than the calculated specific conductivity.

The analysis of the ultrafiltrate shows that the strong acid character of the titration curve cannot be attributed to the hydrochloric acid present in the sol. This resemblance with strong acid disappears however when the titration is carried with higher alkali concentrations and up to alkaline regions. Curve 5 (Fig. 5) is typical of a weak acid. The final rise in pH has not been attained though the alkali added would neutralise an amount of acid forty times the total acid given in Table II.* The particles begin to dissolve and this is responsible for the strong buffer action. The adsorption of the alkali or the dissolution of layers of the particle would both explain the peculiarities of many of the conductometric titration curves in the alkali region. They also explain how local changes in concentration simulating steps of dissociation of weak acids may be produced by the disappearance of the alkali by such reactions unless the stirring is vigorous enough to produce a more uniform reaction. The conductometric titration curve 3′ with barium hydroxide on closer examination shows a departure from the slopes of that of a strong acid. For the sake of comparison the theoretical curves for the neutralisation of a strong acid (hydrochloric acid) by the same alkali is given in dotted lines.

The conductometric titration curve resembles, to a certain extent, the neutralisation of a mixture of a strong and a weak acid, the strong acid in this case being hydrochloric acid. The concentration of the strong acid component of the mixture can be calculated from the point of intersection of the salt line of this acid and the initial straight portion of the curve produced. The concentration thus calculated is, however, very much larger than the total acid of the ultrafiltrate (curve 6) which contains all the free hydrochloric acid.

*More detailed investigations in this direction are in progress.

An initial straight portion followed by a rounding of the curve near about the minimum point is characteristic of the conductometric titration curve of a moderately strong acid. The resemblance disappears however when the titration is carried to alkaline regions, the sharp rise following a round portion being absent. In fact the difference in the slope of the titration curve of the sol after the minimum point has been passed from that of the theoretical curve is even more pronounced in the alkaline than in the acid region. It is interesting to find a similar flattening of the potentiometric titration curve after the sharp rise corresponding with the minimum point of the conductometric titration curve has been passed. A surface reaction in which the alkali is used up is indicated by both sets of curves.

Sol E, on the whole, behaves as a strong acid though not as strong as hydrochloric acid of comparable concentration. The strong acid character is revealed by titration with dilute alkalis only while the titration curve with a strong alkali indicates a weak acid character. The high ratio of free to total acid indicates a strong acid character.

Sol F

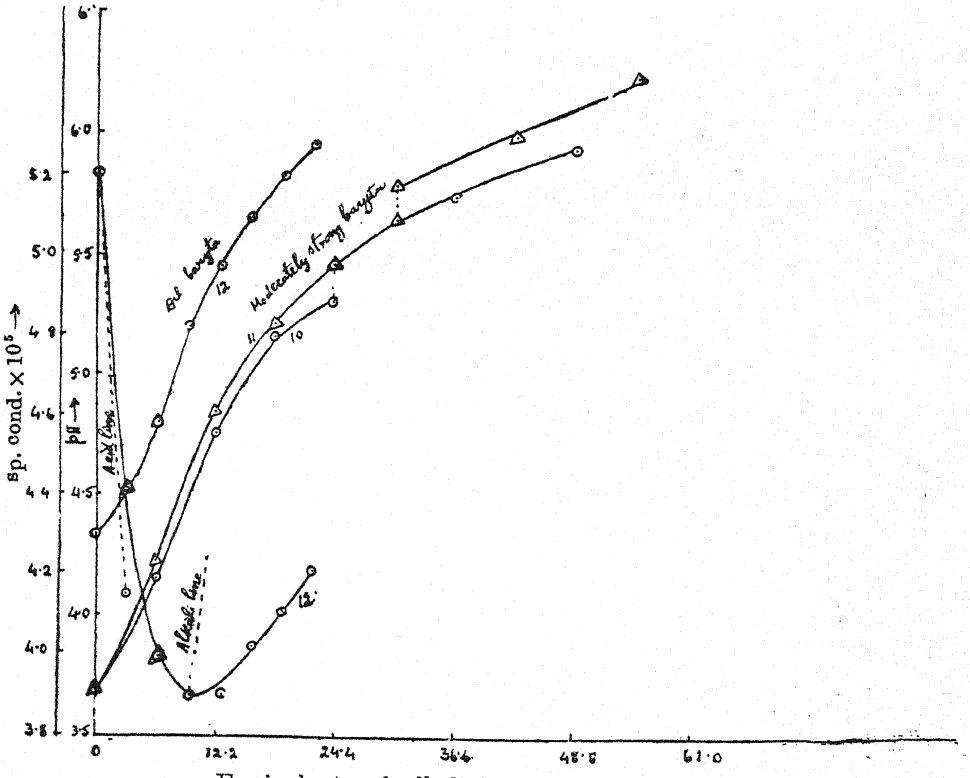
Sol F with lower specific conductivity and hydrogen-ion concentration was next used. The results are given below in Table III.

TABLE III

SiO_2 in grm.-mol. per litre	Total sodium in normality $\times 10^4$	Total chlorine in normality $\times 10^4$	Total acid in normality $\times 10^5$	Free acid in normality $\times 10^5$
0·1440	1·10	1·00	7·8 (curves 12 and 12′)	8·1
0·1440	1·05	0·97		

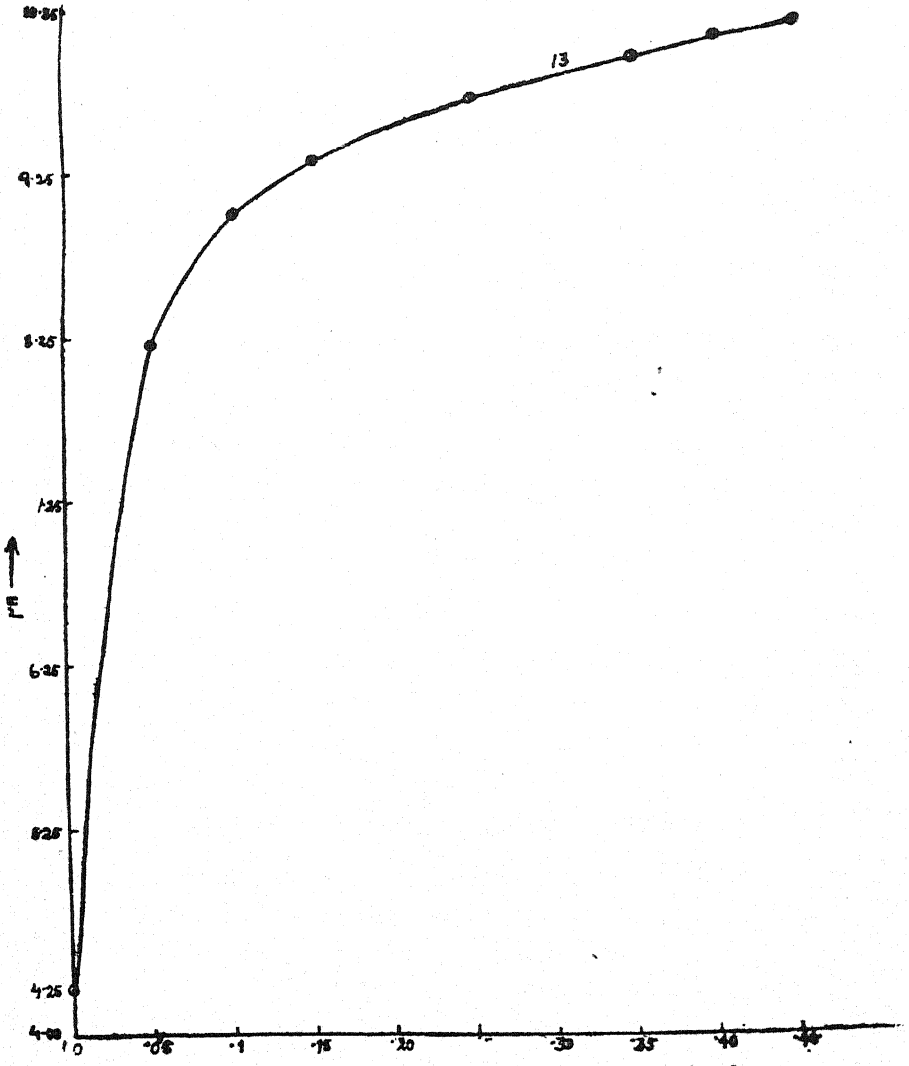
The potentiometric curve 12 (Fig. 8) with dilute baryta resembles that of an acid of medium strength. The conductometric titration curve 12′ gives evidence of buffer action (compare sol E) near about the minimum point. Though not so suitable for calculation of the total acidity for reasons already explained, it gives roughly the same value as curve 12. With an alkali of moderate strength the strong acid character becomes less pronounced (curves 10 and 11) and with more concentrated alkali the curve (curve 13, Fig. 9) becomes typical of a weak acid (compare sol E). The small quantity of the acid involved in the titration and the dissolution of the particles with more concentrated alkali is responsible for the 'weak acid' type of the curve. The initial portions showing a strong acid character would only be observed with dilute alkali. The pH 4·29 from the average e.m.f. of the four measurements which agree between themselves (excluding the two values 3·69 given in Table I) indicates a low dissociation, but if these values

are included in calculating the average, a pH 4·09 is obtained in agreement with the total acidity. As no measurement was made with the quinhydrone electrode no reliance can be placed on this agreement.



Equivalents of alkali in 40 c.c. of sol $\times 10^6$.

Fig. 8. Sol F.



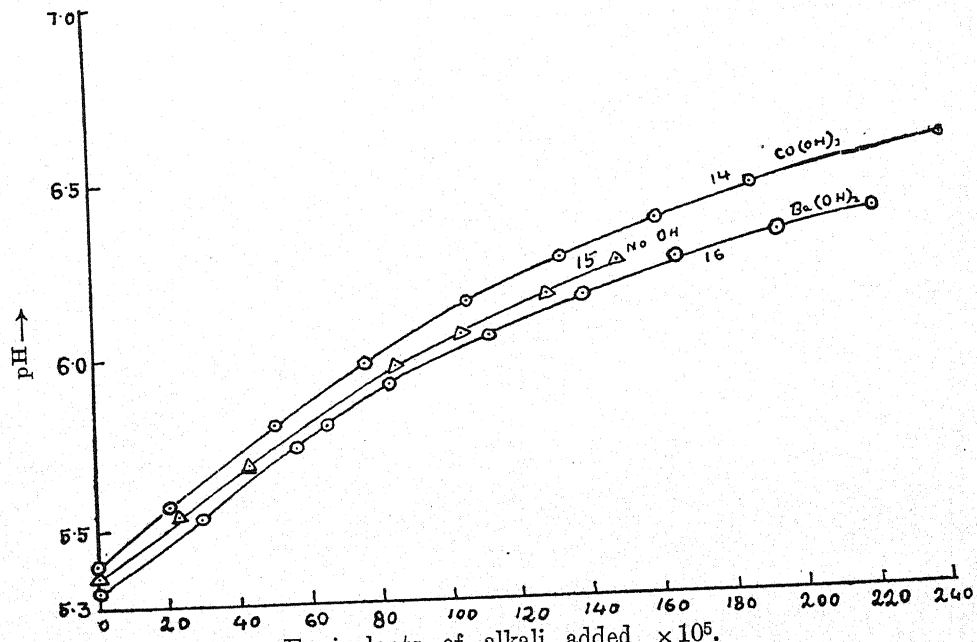
c. c. of NaOH (1·19*N*) added to 40 c. c. of sol.

Fig. 9. Sol F.

The titration of the sol was continued over three days. In agreement with previous observations the initial pH observed next day is always slightly larger than the final equilibrium pH of the previous day.

Sol G

The reproducibility was not satisfactory for sol F. Sol G is more dilute than sol F. Its specific conductivity was about $1{\cdot}5 \times 10^{-5}$ mhos (Table I) and the total acid about 1×10^{-5} grm. ions of H^+ per litre (*vide* curves 14, 15 and 16, Fig. 10). The reliability of the measurements for such sols has an additional interest in relation to the work on suspensions of similar pH by some soil chemists. The curves with dilute sodium, barium and calcium hydroxides are similar and indicate an acid of medium strength. No definite breaks occur. The slopes of the curves indicate (see later) that adsorption or surface reaction plays a proportionately larger part than the neutralisation of hydroxyl ions by hydrogen ions. The free acid ($2{\cdot}4 \times 10^{-6}$ grm. ions of H^+ per litre, calculated from the average pH given in Table I) appears to be considerably smaller than the total acid but the comparison loses its significance for the above reason and the wide variation in the pH values. The titration curve of this sol with strong alkali resembles those of sols E and F and shows a weak acid character. The experimental difficulties are still more pronounced in agreement with the very low concentration of the acid. The results on the whole are of interest as they show that besides a neutralisation process an adsorption or dissolution process should be considered.



Equivalents of alkali added $\times 10^5$.

Fig. 10. Sol G.

Sol H and its dilutions

The preceding results show that sols having a pH below five give more reliable data. Sol H comes within this range. The results are given in Table IV.

TABLE IV

	Conc. of SiO_2 in grm-mol. per litre	Total acid $\times 10^5$ in normality	pH	H ion conc. $\times 10^5$ (free acid) in normality	Free acid / Total acid	Sp. cond. in mhos $\times 10^5$	Total chlorine $\times 10^5$
Sol *H* . .	0·233	14·4 (curves 18 & 18′ with NaOH)	4·02 (Pt_{H_2})				
	0·233	14·4 (curve 19 with NaOH)	4·10 (Q_H)				
		15·5 (curves 20 & 20′ with $Ba(OH)_2$)	average 4·06	8·7	0·57	8·91	5·0
		16·9 (curve 22 with dil. NaOH)					
		average: 15· 3					
Ultrafiltrate of sol H		2·24 (curves 23 & 23′)				2·51	5·00

TABLE IV (*a*)

Dilutions of sol H

	Total acid $\times 10^5$ in normality	pH	H ion conc. $\times 10^5$ (free acid) in normality	Free acid / Total acid	Sp. cond. in mhos $\times 10^5$	Total acid $\times$ dilution
Sol H/2 . . .	7·2 (curve 25; Fig. 14)	4·16 (Pt_{H_2})				
		4·30 (Q_H)				
		average: 4·23	5·8	0·80	4·6	14·4
Sol H/5	3·1 (curves 26 and 26′; Fig. 15)	4·56, 4·61, 4·63 (Q_H)				
		average: 4·60	2·5	0·81	2·1	15·5
		4·09 (Pt_{H_2})				
Sol H/10	1·6 (curves, 27 and 27′; Fig. 16)	4·67 (Q_H)	2·0		1·1	16·0
		4·14 (Pt_{H_2})				
Sol H/20	0·8 (curves 28 and 28′; Fig. 17)	4·91 (Q_H)				
		5·09 (Pt_{H_2})				
		average: 5·0	1·0	1·00	0·56	16·0

The total acid of the ultrafiltrate (curve 23Fig ;11) is again about sixteen per cent of that of the sol. It appears that the method of preparation of the sol, which involves a neutralisation of the sodium silicate by the same proportion of hydrochloric acid and dialysis till no trace of chlorine is found in the dialysate with silver nitrate, leaves almost the same proportion of hydrochloric acid in the sol. The chlorine-ion concentration of the ultrafiltrate is again the same as that of the sol, *i.e.*, the whole of the chlorine comes out in the ultrafiltrate.

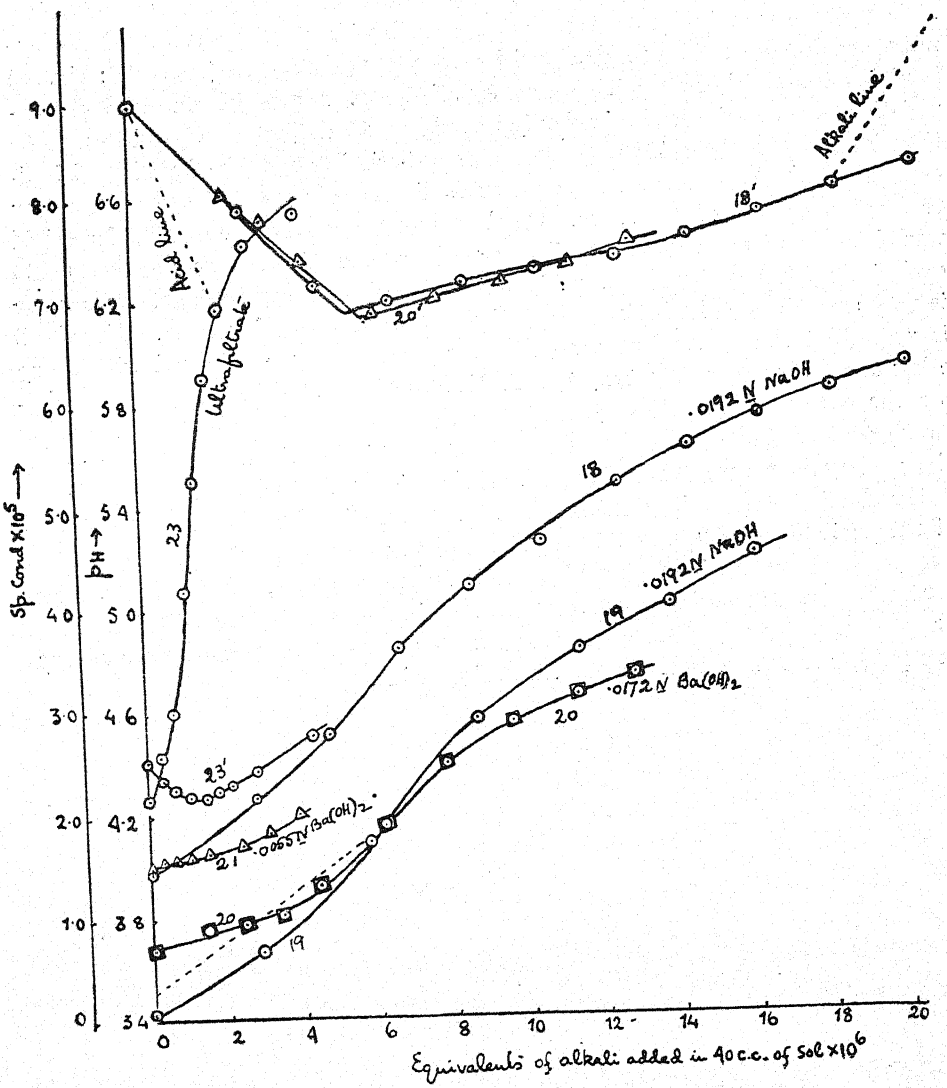


Fig. 11. Silicic acid sol H.

From Fig. 11 it appears that the curves are fairly reproducible. The two curves for sodium hydroxide (curves 18 and 19) are parallel for reasons discussed earlier. The conductometric curves for both sodium and barium hydroxides show a change in curvature a little after the turning point has been passed. A reaction with the alkali is clearly indicated (compare sol E). The potentiometric curve with barium hydroxide is different from that with sodium hydroxide. The former reacts more strongly than the latter, the differences being particularly pronounced in the initial stages where a strong buffer action is shown with barium hydroxide. It is interesting to find, however, that the total acidities with the two alkalis are not much different. The total acid with very dilute caustic soda (curve 22 ; Fig. 12) is higher than with moderately strong caustic soda. The initial convexity characteristic of strong acids is obtained only with dilute alkali (curves 18 and 20). The titration curve 24 (Fig. 13) with strong alkali is similar to that of sol E and indicates a weak acid character. The particles dissolved in the region corresponding to the flat portion of the curve. Conductometric titration curve 22 for sol H was obtained with a Vreeland oscillator as the source of alternating current (1000 cycles) and a Leeds Northrup set of Curtis Coil resistances and bridge. The curve is very smooth and the horizontal portion of the curve is of interest. The alkali is very dilute. The conductometric curves resemble those observed with many other sols [Compare sols A/2, A/6, A/11, Mukherjee *et al.*, 1934]. In considering the curvature the observed slope should be compared with the theoretical slopes (dotted lines) for neutralisation of hydrochloric acid under similar conditions. The departure from the theoretical slope is, as with sol E, more pronounced in the alkaline than in the acid region. At high alkali concentrations a dissolution of the particles requires to be considered. When the total acid concentration is large, the convexity in the acid region is less pronounced, possibly because the neutralisation process forms the major proportion of the reaction with the alkali. The slopes, compared with the theoretical slopes, show more definitely the adsorption process near and after the neutralisation point.

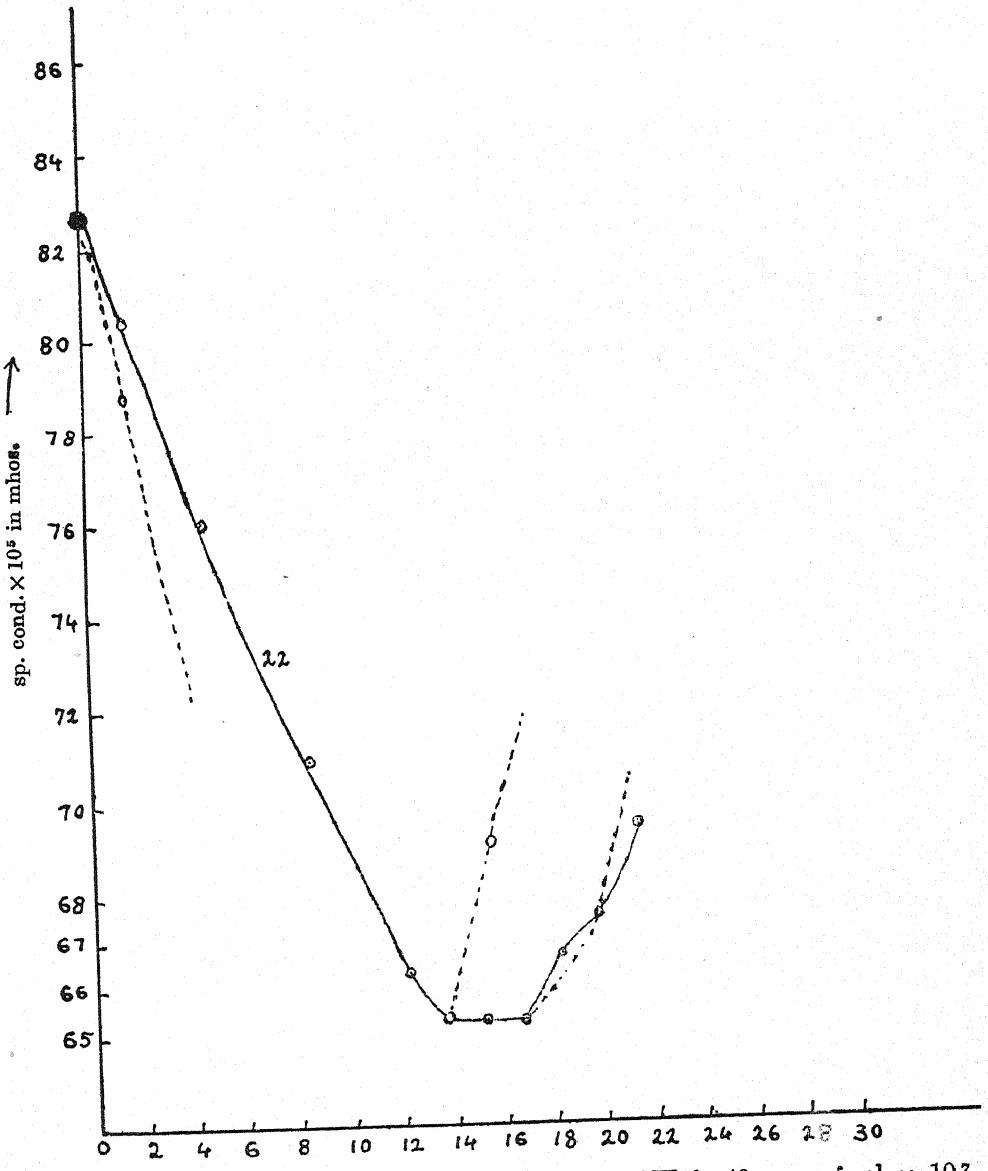
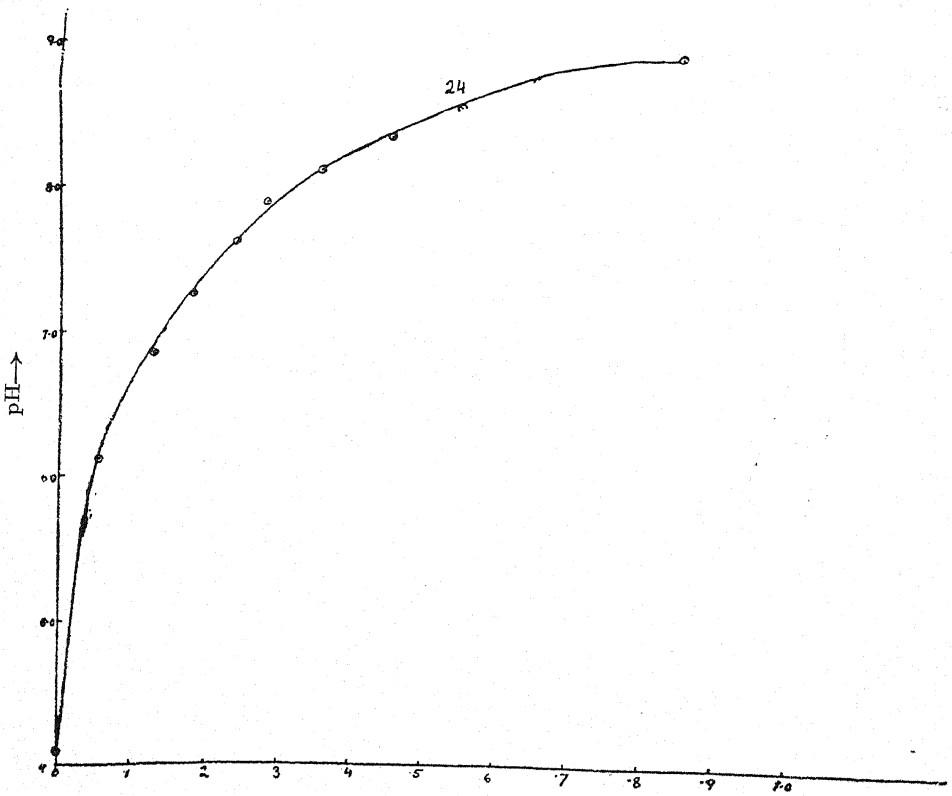

86
84
82
80
78
76
74
72
70
68
67
66
65
sp. cond. × 10⁵ in mhos.
22
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Equivalents of alkali (0·00695N NaOH) in 40 c. c. of sol × 10⁷

Fig. 12. Sol H.

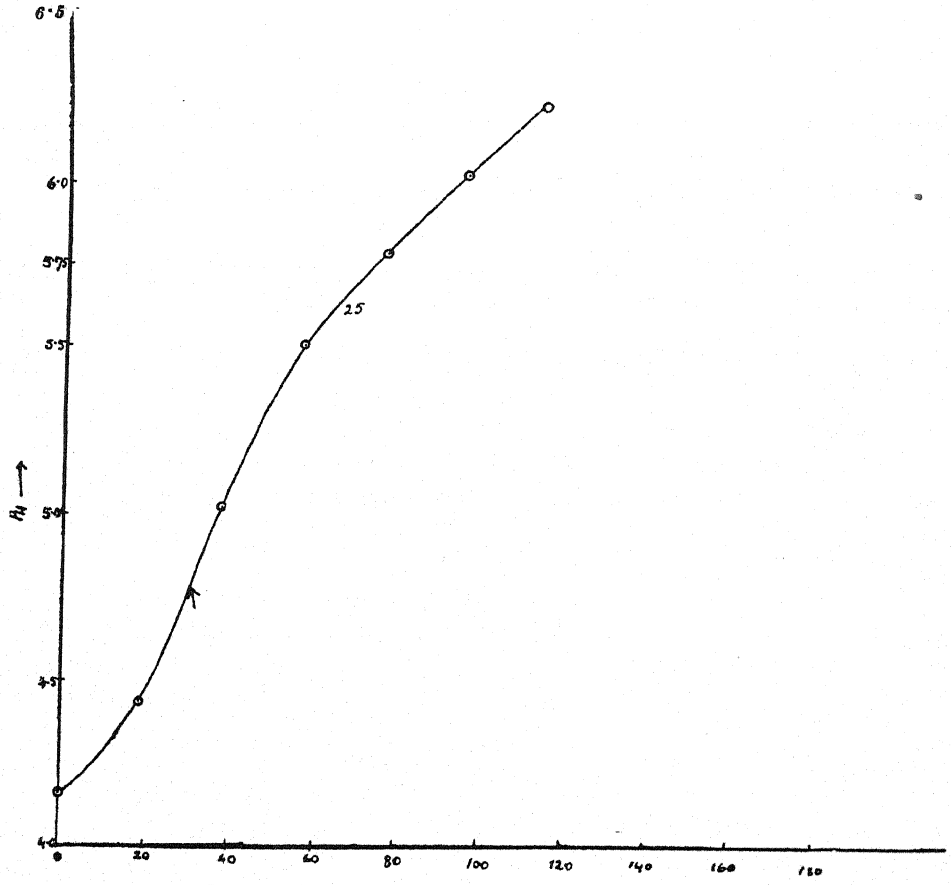


c. c. of strong NaOH (0·34*N*) in 40 c. c. of sol.

Fig. 13. Sol H.

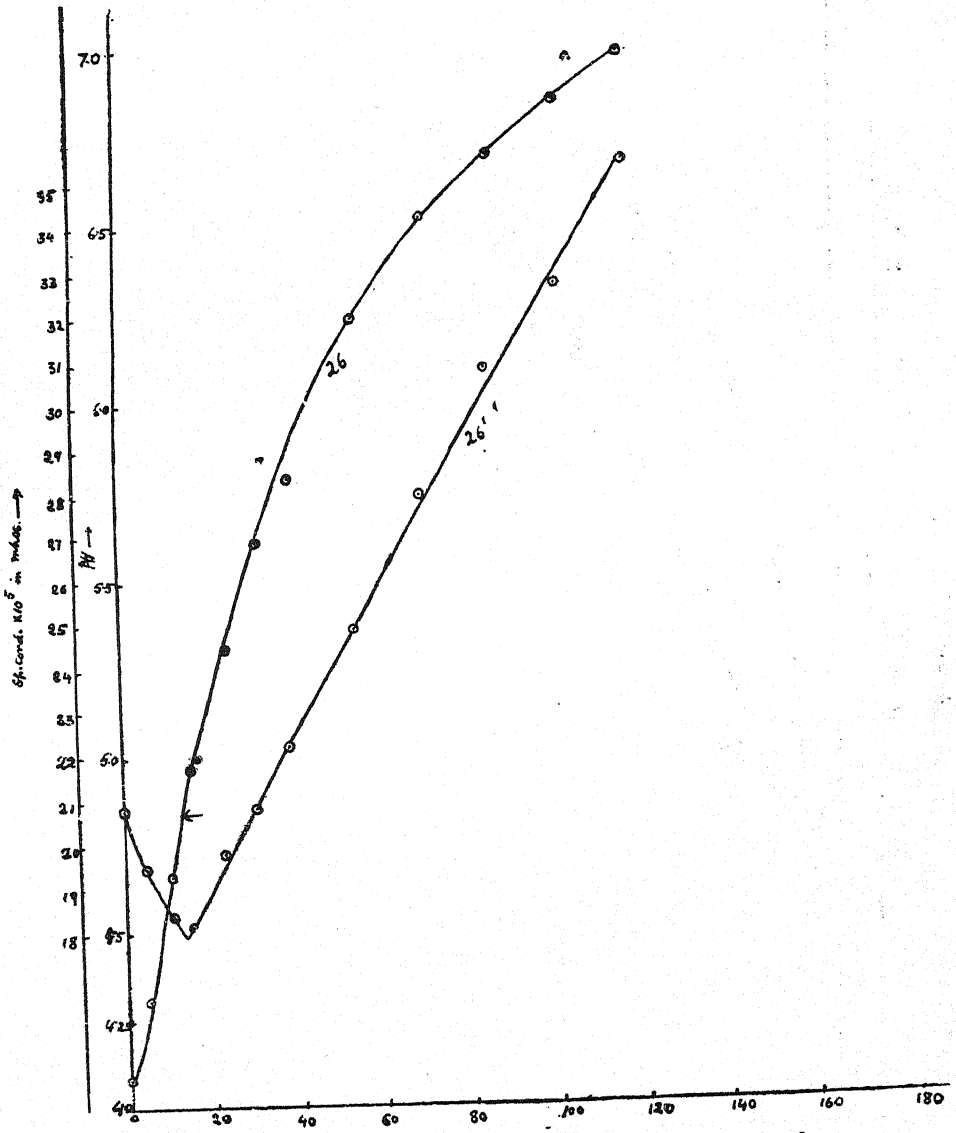
The total acid decreases almost proportionately with the dilution up to sol H/5. With sols H/10 and H/20 the total acid calculated from the curve is liable to larger percentage errors in view of the extremely small quantities of the acid present. The same alkali was employed in the titration of the different dilutions of sol H and the manner of addition was also nearly the same as previous experience with silicic acid sols [Mukherjee *et al.*, 1934] indicates that the total acid depends to some extent on these factors. Thus the total acid for sol H is larger with dilute alkali than with more concentrated alkali. The change in pH due to the same quantity of the alkali being added, $\frac{\delta pH}{\delta c}$, is comparatively large with the more dilute sols H/10 and H/20 and the location of the exact point of inflexion between the two points between which $\frac{\delta pH}{\delta c}$ is maximum is more difficult. The total acid changes regularly on dilution as is shown by the agreement between the ' average ' total acidity of sol H and the total acid of the diluted sols multiplied by their dilution. This regularity does not preclude the existence of an adsorption process. The amount of alkali adsorbed perhaps varies proportionately with the dilution, depending as it should on the particle concentration and the equilibrium concentration of the alkali.

The specific conductivity changes very regularly with the dilution and this illustrates the satisfactory nature of the arrangements. The free acidities for sols H and H/2 are fairly reliable in view of the good agreement between the average values from the hydrogen electrode and the value from the quinhydrone electrode. The free acidities of sols H/5, H/10, and H/20 obtained from the quinhydrone electrode have been taken to be more reliable and have been used for calculating the degree of dissociation, but the absolute values cannot be relied upon to the same extent as for sols H and H/2. The sol H is about sixty per cent dissociated. The strength of the acid character would be better understood by plotting log r against log c where r is the ratio of free to total acid of the different dilutions of the sol and c their respective total acid concentrations. A similar plot for hydrochloric acid solutions of comparable concentrations would facilitate the comparison.



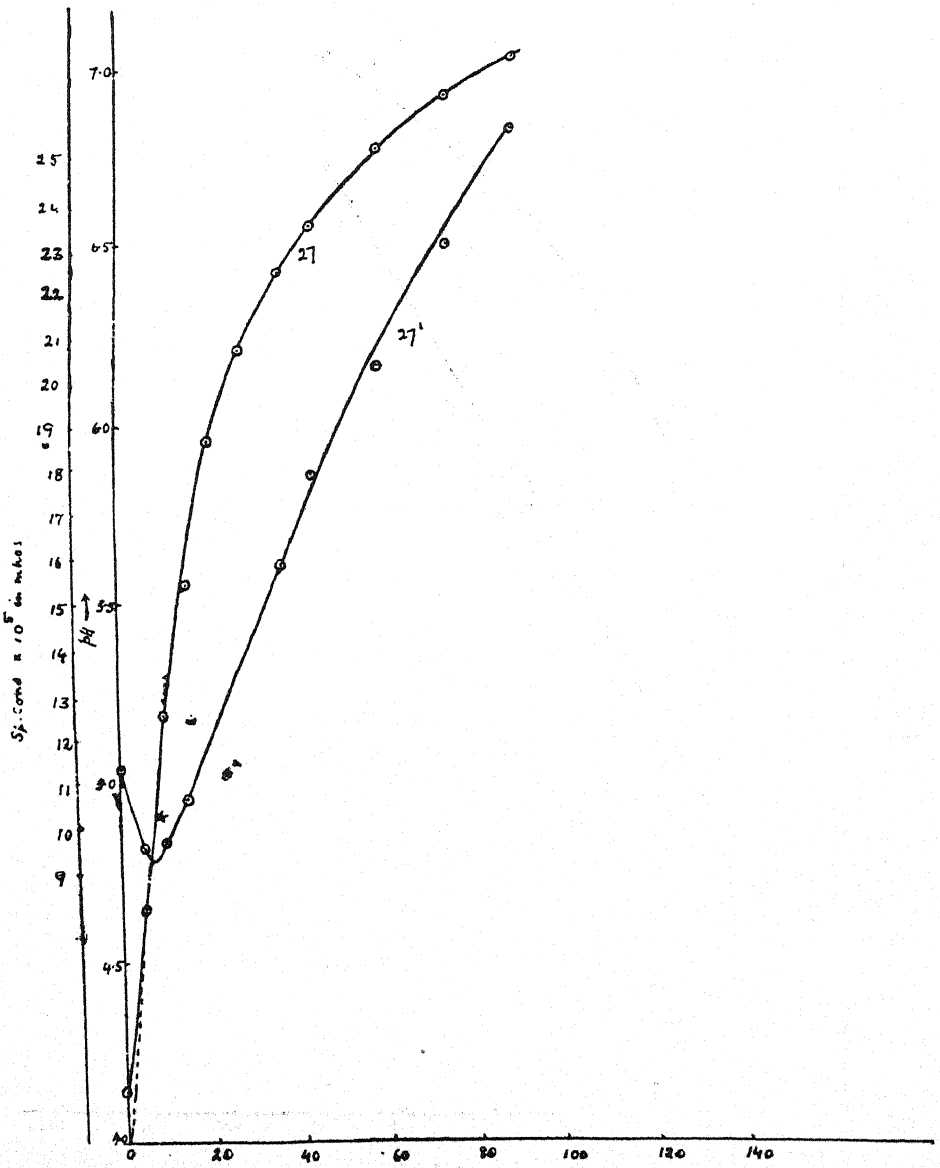
Equivalents of alkali $\times 10^7$ in 40 c. c. of sol.

Fig. 14. Silicic acid sol H/2.



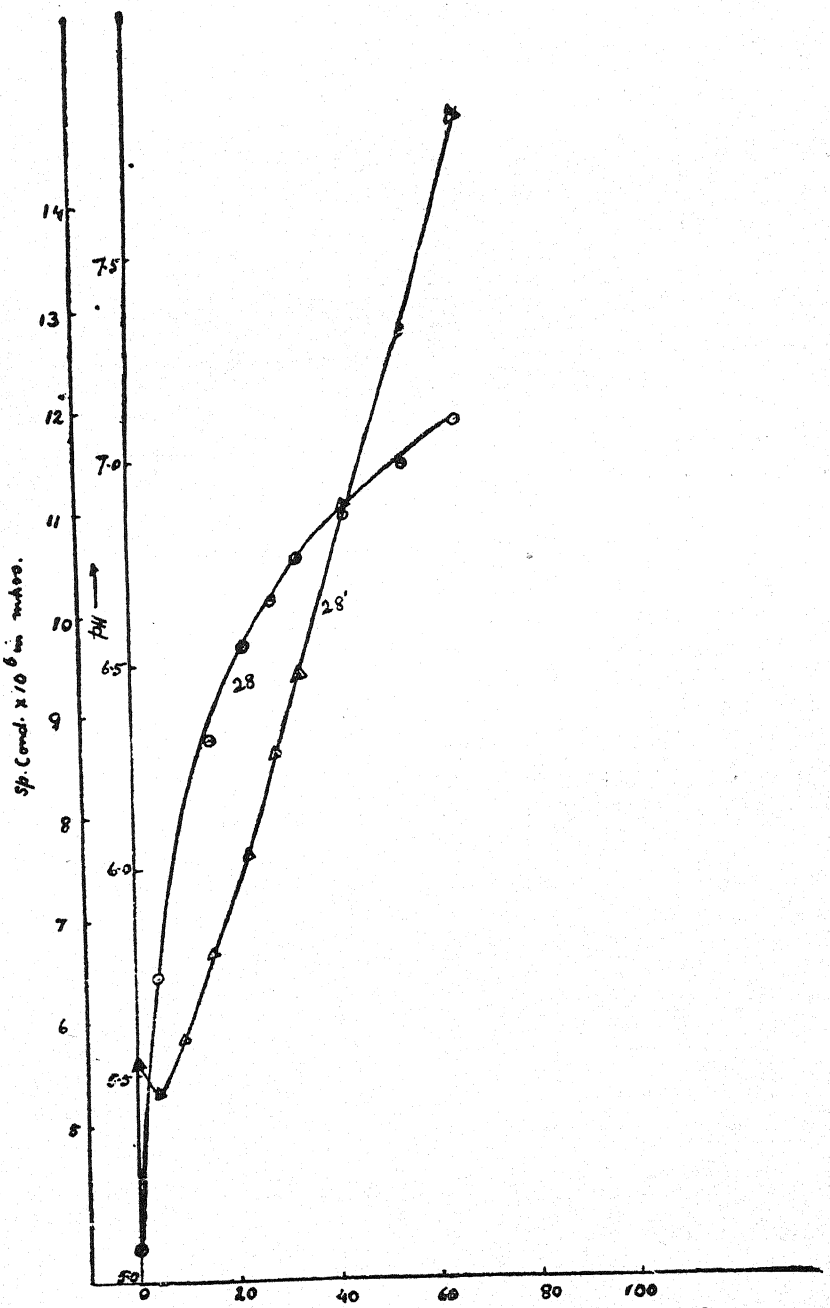
Equivalents of alkali (NaOH) $\times$ 10^7 in 40 c. c. of sol.

Fig. 15. Silicic acid sol H/5.



Equivalents of alkali (NaOH) × 10^7 in 40 c. c. of sol.

Fig. 16. Silicic acid sol H/16.



Equivalents of alkali (NaOH) × 10^7 in 40 c. c. sol.

Fig. 17. Silicic acid sol H/20.

Silicic acid sol I

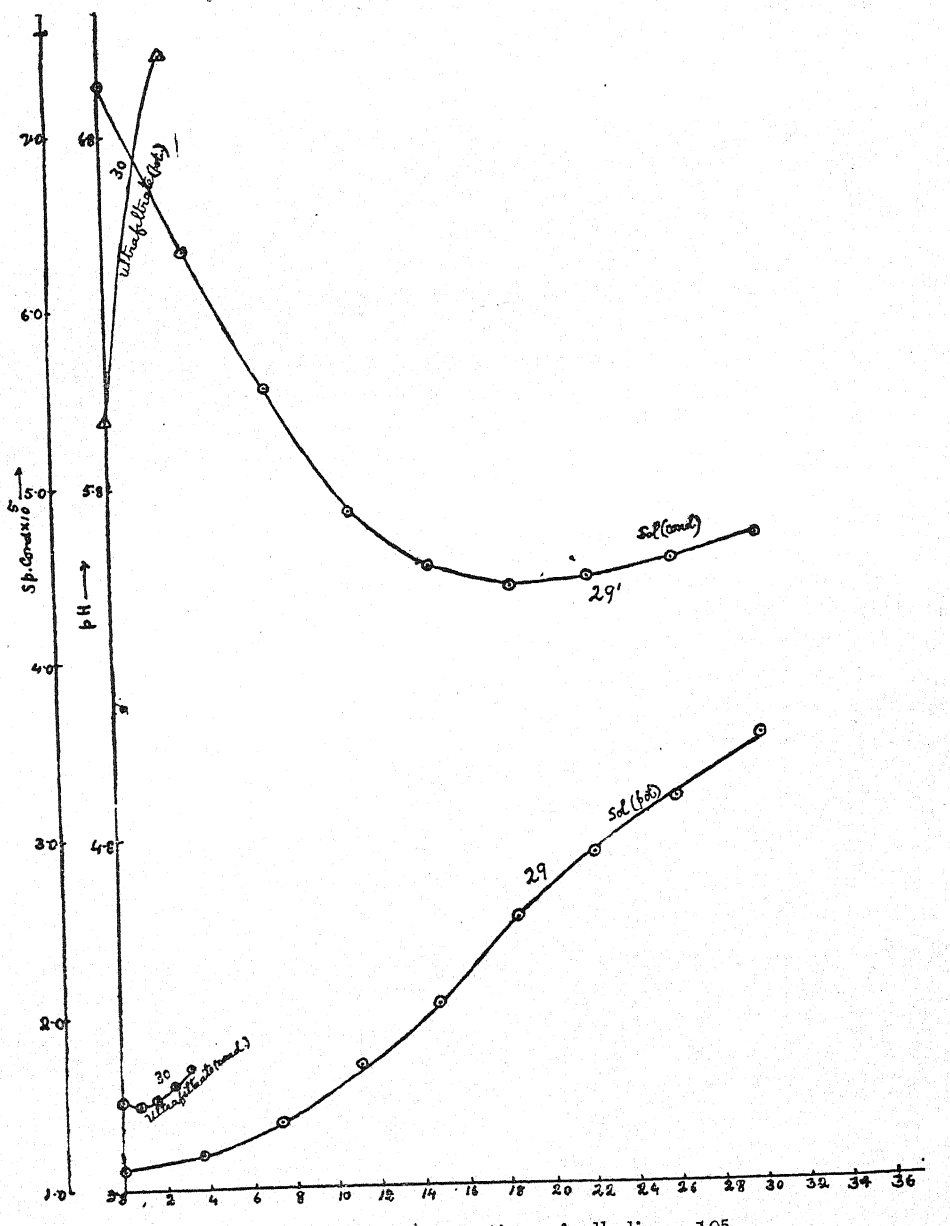
Dissolved hydrochloric acid contributes an appreciable fraction (15 per cent) to the observed total acids of sols E and H. It was thought that prolonged dialysis might remove these last traces of hydrochloric acid from the sol. Accordingly sol I was dialysed for a much longer interval of time against repeated changes of conductivity water towards the end of the operation. The results with this sol. are given below :

Sol I

TABLE V

	Grm—mols. of SiO_2 per litre	Total chlorine	Sp. cond. in mhos $\times 10^5$	pH	H-ion conc.	Total acid
Sol I	0·235	Negligible	7·3	3·85	$1·41 \times 10^{-4} N$	$16·6 \times 10^{-5} N$ (curves 29 and 29′; Fig. 18)
Ultrafiltrate of sol I .	...	Negligible	1·52	6·2	$6·31 \times 10^{-7} N$	$0·8 \times 10^{-5} N$ (conductometric curve 30′ ; Fig. 18)

The analysis of the ultrafiltrate shows that dissolved acids contribute a very negligible fraction (curves 30 and 30′ ; Fig. 18) to the observed total acid. The chlorine-ion concentration is nil and the ultrafiltrate is practically neutral. The titration curve 29 (Fig. 18) with sodium hydroxide indicates a moderately strong acid character of the sol ; the conductometric titration curve 29′ is similar to that of sol H. Sol I thus presents the same broad features as sol H.



Equilibrium concentration of alkali × 10^5.

Fig. 18. Silicic acid sol I.

In a recent paper Rabinowitch and Kargin [1935] have expressed the view that the acid character attributed by previous workers to silicic acid sols should really be referred to the presence of dissolved acids in the sol. In support of their view they quote some experimental results with silicic acid sols purified by prolonged electrodialysis which show that these sols showed practically neutral reaction and had conductivities comparable with that of pure water. As pointed out by Valko [1935] the above behaviour of Rabinowitch's sols was due to their extremely low silica contents. Both silicic acid sols H and I are about sixty times more concentrated than Rabinowitch's sols. As already pointed out the small total acid of the ultrafiltrate of sol I in particular, shows that the strong acid character of this sol cannot be due to free acids present in the sol. Also unpublished work from this laboratory with sufficiently concentrated electrodialysed silicic acid sols shows that these sols have the same order of magnitude of total acidities as sols H and I and they present the same broad features.

Basicity of colloidal acids

Colloidal acids have been considered to be polybasic acids in true solution [Pauli and Valko: Elektrochemie der kolloide, 1929]. The titration curves given in this paper bear no evidence of a polybasic character. They are rather typical of monobasic acids. A closer inspection, however, will show that this resemblance is only superficial. The slope of the titration curve of a weak monobasic acid at the point of half neutralisation is independent of the dissociation constant of the acid and is given under certain conditions of titration by Auerbach and Smolczyk [1924].

$$\frac{\delta \text{ pH}}{\delta x} = \frac{1 \cdot 74}{xa}$$

where xa denotes the volume of the alkali required to reach the equivalence point. The theoretical slope calculated from the equation (dotted lines in Fig. 12 ; sol H) seldom agrees with the slope. The discrepancy is more pronounced with the titration curve with barium hydroxide. This shows that the silicic acid sols cannot be treated as weak monobasic acids in true solution. Adsorption by and dissolution of the particles are, on the other hand, sufficient to account for the forms of the titration curves.

"Strength" of silicic acid sols

In Table VI the free and total acids of different silicic acid sols where the value of the free acid could be fairly relied upon have been summarised and "dissociation constants" calculated from them given in the last two columns.

TABLE VI

	Total acid	Free acid	Free acid / Total acid	K_1	K_2
Sol I (curve I, part I, of this series).	0·0034*N*	0·00234*N*	0·688	$5\cdot5\times10^{-3}$ $7\cdot0\times10^{-3}$ $13\cdot2\times10^{-3}$	$5\cdot16\times10^{-3}$
Sol A/2 (curve III, part II of this series) . .	0·0065*N*	0·0059*N*	0·91	$3\cdot56\times10^{-1}$ $3\cdot87\times10^{-1}$	$5\cdot89\times10^{-2}$
Sol A/11 (curve VII, part II, this series) . .	0·00087*N*	0·00084*N*	0·97	$6\cdot11\times10^{-2}$	$2\cdot3\times10^{-3}$
Sol D (curves 1 and 2 . .	0·00009*N*	0·000048*N*	0·53	..	$5\cdot4\times10^{-3}$
Sol E (curve 3) .	0·00030*N*	0·000257*N*	0·85	$3\cdot9\times10^{-3}$ $5\cdot9\times10^{-3}$	$1\cdot75\times10^{-3}$
Sol H . . .	0·000153*N*	0·000087*N*	0·57	$4\cdot6\times10^{-5}$ $1\cdot29\times10^{-4}$	$1\cdot15\times10^{-4}$
Sol H/2 (curve 25).	0·000072*N*	0·000058*N*	0·80	$6\cdot4\times10^{-5}$	$1\cdot9\times10^{-4}$
Sol H/5 (curve 26)	0·000031*N*	0·000025*N*	0·81	$2\cdot56\times10^{-5}$ $3\cdot24\times10^{-4}$	$1\cdot1\times10^{-4}$

Inspite of the strong acid character of the titration curves colloidal silicic acid is often dissociated to the extent of about sixty per cent though the total acid concentration is of the order of 10^{-5} grm. ions of hydrogen per litre. It appears also that the free and total acids do not depend solely on the concentration of the silica.

The 'dissociation constants' K_1 calculated from the following equation have been given in column 5 of Table VI.

$$\mathrm{pH} = \mathrm{p}K_1 + \log \frac{[\mathrm{Salt}] + [A']}{[\mathrm{Acid}] - [A']}$$

The pH corresponding to a given addition of the alkali is obtained from points in the neighbourhood of half neutralisation; the concentration of the colloidal salt, [salt], is taken to be equivalent to the alkali added; the concentration of the unneutralised acid, [acid], is given by the difference of the total acid as obtained from the titration curve and the concentration of the added alkali; $[A']$ denotes the concentration of the anions dissociated from the unneutralised acid and is roughly given by the product of the concentration of the unneutralised acid with the ratio of free to total acids; the concentration of unneutralised undissociated acid is given by [acid] $-[A']$. In view of the high ratio of free to total acids the more simple Henderson equation appears to be inapplicable for calculating the constants. In the last column of table 'dissociation constants', K_2 calculated from the equation $K_2 = \frac{\alpha^2 . c}{1-\alpha}$

have been given. α is given by the ratio of free to total acids and the molecular concentration c by the total acid as obtained from the titration curves, the acid being assumed to be monobasic.

Though conveying an approximate idea of the ' strength ' of silicic acid sols the calculated ' dissociation constants ' are not constants in any strict sense of the term. In a previous paper [Mukherjee *et al.*, 1934] it has been observed that the 'dissociation constants ' of colloidal acids have a fictitious significance because of the indefiniteness, among other things, of the total acid taking part in the reaction. Thus the total acid has been found to depend on the nature and concentration of the alkali (compare the curves with dilute and concentrated alkalis given in this paper), the time allowed for interaction [Mukherjee *et al.*, 1932] and on other factors.

For an interpretation of the titration curve of an acid in relation to the strength of its acid character one need consider, besides the dissociation constant, the solubility of the salt formed and its degree of hydrolysis. The hydrolysis of a salt is expected to be greater with a weak alkali than with a stronger one, and on this ground a titration curve with barium hydroxide should indicate a greater rise of pH than a curve with sodium hydroxide. The titration curves of silicic acid sol H show that this is contrary to our observation. Work from this laboratory with palmitic acid sols show that the titration curve with barium hydroxide shows a strong buffer action which is not shown by the curve with sodium hydroxide. In the case of an acid in true solution its neutralisation by an alkali requires different theoretical treatment according as the salt formed is in solution, or, forms an insoluble precipitate. In the neutralisation of a silicic acid sol with barium hydroxide we are concerned with the adsorption of barium ions on the oppositely charged primarily adsorbed ions on the surface of the colloidal particles whose dimensions are large compared to that of ordinary molecules or atoms. The formation of a secondarily adsorbed layer of barium ions on the surface has to be distinguished from the formation of an insoluble precipitate, *e.g.*, barium silicate. The ' ion pairs ' formed by adsorption may not constitute a distinct phase. The solubility of such a ' salt ' does not convey any clear meaning.

The interaction of ions on the surface of colloidal particles is more readily understood from the standpoint of the adsorbabilities of ions as postulated by Mukherjee [1921]. According to this view the particles of colloidal acids owe their negative charge to the presence on the surface of a fixed layer of primarily adsorbed anions. Corresponding to the number of these ions an equivalent number of oppositely charged ions (in this case hydrogen ions) remain in the liquid near the surface. A certain number of these hydrogen ions may remain fixed on the surface by electrostatic or chemical valency forces forming an electrically adsorbed layer while the remaining hydrogen ions form the ' mobile sheet ' of the double layer. On the addition of an alkali besides the simple neutralisation of

the ' mobile ' hydrogen ions by the hydroxyl ions the cation of the added alkali may displace some of the electrically adsorbed hydrogen ions which can then neutralise further quantities of hydroxyl ions. The hydroxyl ions of the added alkali may be primarily adsorbed by chemical forces with a consequent increase in the negative charge of the particles which can now drag in more cations into the double layer. The greater the adsorption of the cations the more pronounced will be the buffer action. The relative ' electrical ' adsorbabilities of the cations is a function of their valencies, mobilities and states of hydration. On this basis alone the adsorbability of barium ions should be stronger than that of sodium ions and would account for the stronger buffer action of silicic acid sols towards barium hyroxide than towards sodium hydroxide (compare sol H).

Action of neutral salts on colloidal acids

The interaction of soil with neutral salts is of fundamental interest to soil chemistry. A distinction should be made between the sol and the gel state. In the latter case any displaced hydrogen ion whether it comes from the mobile sheet of the double layer or from the primarily or secondarily adsorbed layers will always show an increase in the acidity of the supernatant water. In the case of the sol a free hydrogen ion in the mobile sheet so displaced by a cation of a salt of a strong acid will not alter the pH unless the activity coefficient of the hydrogen ion in the mobile layer is different from what it is in the intermicellar liquid. An increase in the hydrogen ion concentration denotes displacement of hydrogen ions from the primarily or secondarily adsorbed layer. Analyses of the ultrafiltrate and estimation of the total acidity in the presence of the salt would afford valuable information of the interaction.

To sols H and I were added gradually increasing amounts of neutral solution of potassium and barium chlorides and the resulting changes in pH are given below.

Table VI

	Total acid as obtained by titration with alkalis (no neutral salt being added)	Initial pH without addition of salt	pH after addition of salt	Conc. of salt added
Sol H	$15{\cdot}2\times10^{-5}N$	4·0	3·3 (curve 31)	0·09 N $BaCl_2$
		4·05	3·70 (curve 32)	0·09 N KCl
Sol I	$16{\cdot}6\times10^{-5}N$	3·60	3·20 (curve 35)	0·013 N $BaCl_2$
		3·75	3·58 (curve 36)	0·013 N KCl

The curves 31 and 32 (Fig. 19) for sol H and curves 35 and 36 (Fig. 20) for sol I show that barium chloride decreases the pH to a greater extent than potassium

chloride. For comparison the pH of a hydrochloric acid solution of approximately the same original pH as the sol when titrated in a similar manner, is given in curves 33 and 34 (Fig. 19). The increase in the hydrogen-ion concentration on the addition of barium chloride is about 300 per cent for sol H and 150 per cent for sol I. In fact more acid appears to come out on the addition of barium chloride than the total acid obtained by titration with alkalis. The results are significant in that they bring out prominently the indefinite character of the total acid and the important part played by the cation in reactions in colloidal acid systems. Analysis of the ultrafiltrate and estimation of the total acid in the presence of the salt would afford valuable information regarding the interaction. The estimation of the total acids of silicic acid sols in the presence of the salt is difficult as the sols rapidly set under these conditions. The following table will show that the hydrogen-ion concentration and the total acid of the ultrafiltrate of sol I—the ultrafiltrate being obtained after addition of barium chloride to the sol—are considerably higher than those of the ultrafiltrate and somewhat grater than that of the pure sol. This indicates that the barium ions, in consequence of their stronger electrical adsorbability, displace some of the electrically adsorbed hydrogen ions into the intermicellar liquid.

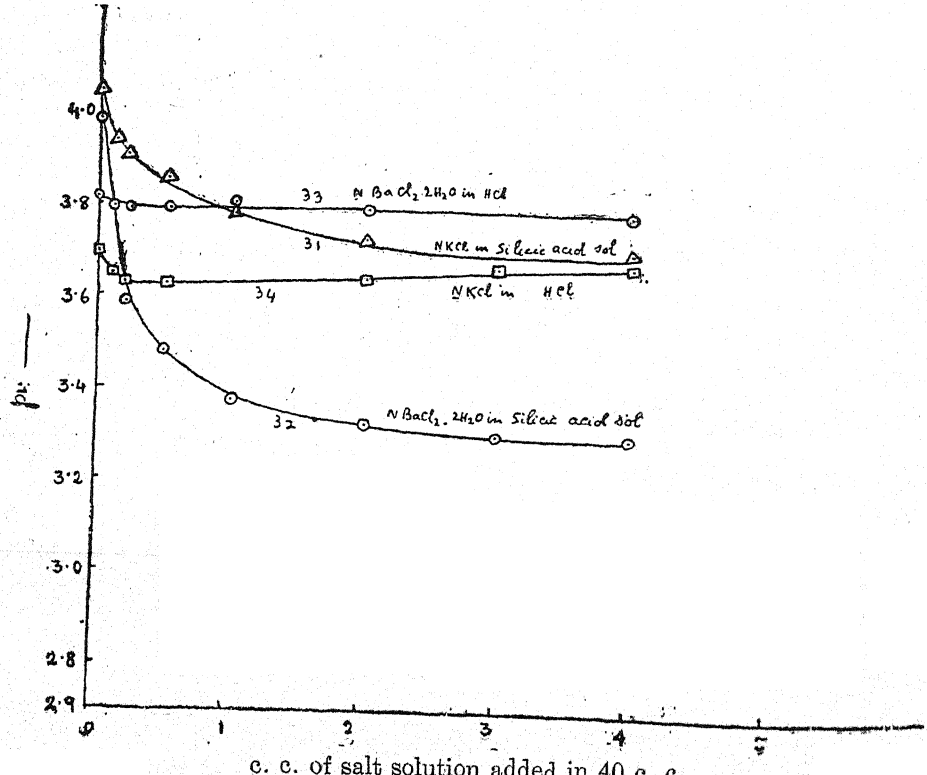
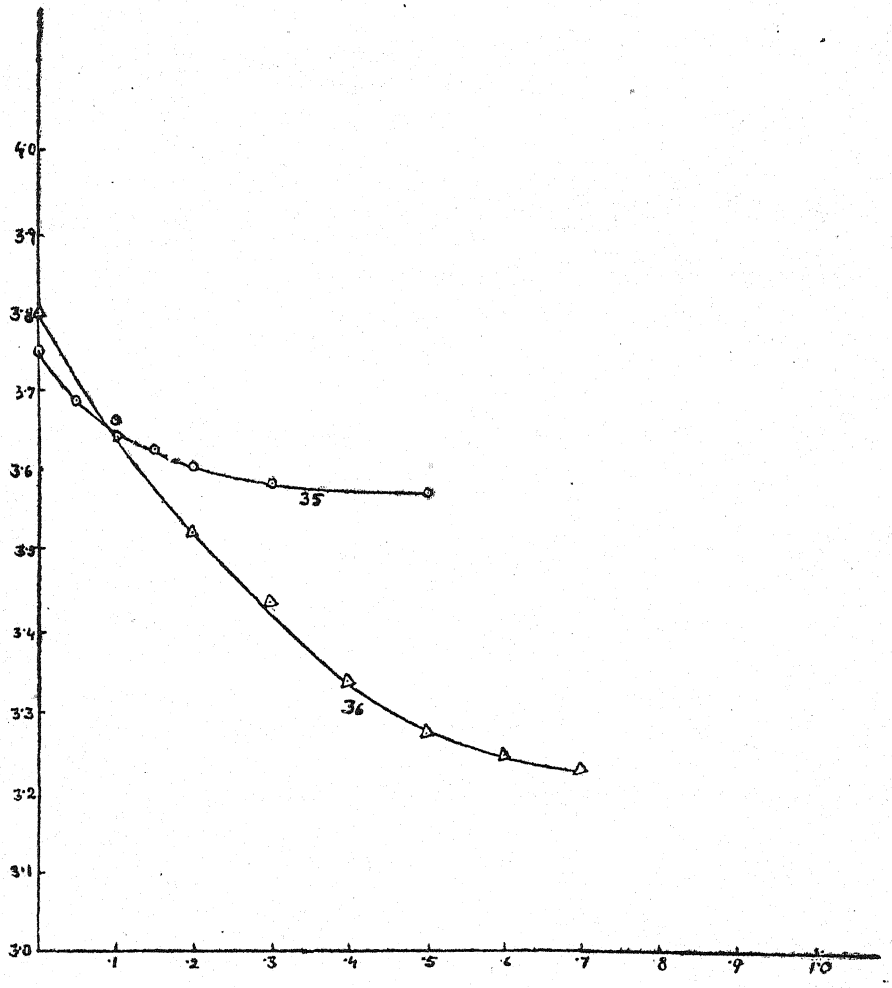


Fig. 19. Sol H.



c. c. of normal salt solution added to 40 c. c. of sol.

Fig. 20. Silicic acid sol I.

	pH	H^+ ion conc. in normality	Total acid	Conc. of $BaCl_2$
Ultrafiltrate of sol I without addition of $BaCl_2$ to the sol . . .	6·2	$6·31 \times 10^{-7}$	$0·8 \times 10^{-5} N$	*nil*
Ultrafiltrate (sol I with $BaCl_2$) . . .	3·98	$1·05 \times 10^{-4}$	19×10^{-5}	$0·059 N$

A detailed discussion of this aspect will be taken up in a future publication.

Summary

The electrochemical properties of a number of silicic acid sols have been studied with special reference to the nature of their titration curves with solutions of alkalis and neutral salts.

The titration curves with dilute alkalis point to a strong acid character while with more concentrated alkalis a weak acid character is revealed, the particles dissolving in the higher alkaline regions. Analyses of the ultrafiltrates of the sols show that their strong acid character cannot be wholly attributed to dissolved acids present in the sol.

The titration curves give evidence of surface reactions distinct from the simple neutralisation of an acid by a base. Adsorption by and dissolution of the particles can, in most cases, account for the forms of the titration curves.

Neutral salts liberate acids from the sols to an extent depending on the ' electrical adsorbabilities ' of the cations.

The authors' thanks are due to the Imperial Council of Agricultural Research for enabling one of them (R. P. M.) to carry out this work as a Research Assistant and for other facilities.

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ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY

PART V. TITRATION CURVES OF CLAY ACIDS

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(With eleven text-figures)

The 'hydrogen clay' obtained by replacing the exchangeable cations of soil by hydrogen ions possesses acidic properties. Several points of view have been suggested to explain the acid character. On titration with alkalis the clay acids have been found by Bradfield to yield titration curves resembling those of monobasic acids [Bradfield, 1927] while some authors have attributed a polybasic character to these acids from observations of more than one 'break' in the titration curves. According to Bradfield the curves, though not as steep at the inflection point as those of simpler acids, show well-defined endpoints using both conductometric and potentiometric methods.

Against a true acid character of the clay acids is the fact that their titration curves with different strong bases can scarcely be superimposed. Oakley's observation [Oakley, 1927] that superposition is possible provided that the titrations are carried out in the presence of semi-normal solutions of the chlorides of the corresponding bases is interesting as it constitutes a difference in behaviour of the clay acids from that of acids in true solution. The discrepancy regarding the non-equivalence of different baseshas been referred to complex side reactions*e.g.* hydrolysis arising from the relatively large ratio of soil to the reacting base as obtain in the usual procedure of electrometric titrations of weak acids when the acid is taken in the titration vessel and the alkali added from outside [Bradfield, 1924]. Using the reverse procedure in which the hydrogen clay was added to the base Bradfield obtained the same total acidity with different bases. Bradfield's method as also the one adopted by Hissink [1925] in which the soil is placed in excess baryta and the amount of alkali reacted upon obtained by titrating the excess alkali, have been criticised by Schofield [1931] on the ground that at high pH's

in the presence of alkali cations soluble aluminates and silicates are formed while with the alkaline-earth cations the aluminates and silicates being insoluble form a layer over the colloidal clay particles. The instability of the particles in the alkaline region thus makes it difficult to obtain, by the above methods, definite information regarding their acid character. The difficulty in finding the endpoint of titration has led to the suggestion that the process is essentially one of adsorption in which both the cations and the hydroxyl ions are adsorbed. This view seems to be supported by a large amount of experimental evidence among which mention may be made of Pennyquick's observation [1930] that hydroxyl ions disappear by a sort of chemical combination with acid groups on the surface as also of Mattson's experiments [1928] showing that when a 'hydrogen clay' is treated with calcium hydroxide the gain in weight of the oven-dried clay corresponds to an adsorption of calcium hydroxide as a whole and not to a simple replacement of hydrogen ions by calcium ions.

In previous papers of this series [Mukherjee *et al.*, 1931, 1932 and 1934] the electrochemical behaviours of individual colloidal ingredients of the soil —acidic (silicic acid sols) and basic (aluminium hydroxide sols)—, have been discussed in the light of experimental results concerning mainly with their titration curves with acids and alkalis. Work with actual clay acids had been deferred pending these investigations with the simpler systems as it was thought that the results obtained thereof would be helpful towards a better understanding of the nature of the more complicated reactions responsible for soil acidity. This preliminary work with pure systems has now sufficiently advanced to justify investigations with actual clay acids. In the present paper the features presented by a number of clay acids isolated from different soil samples have been discussed with special reference to their titration curves with alkalis and solutions of neutral salts.

Experimental

Method of preparation of the clay acids.—The soil was air-dried, powdered and sieved as usual. The organic matter was destroyed by exhaustive treatment with six per cent hydrogen peroxide after which the material was vigorously shaken for several hours with a large volume of water in a motor-driven shaker. The coarse suspension thus obtained was allowed to settle for two to three days. On centrifuging the supernatant liquid with a Sharple's Supercentrifuge a fine stable suspension was obtained. The corresponding 'hydrogen clay' was prepared either by electrodialysis or by leaching with dilute hydrochloric acid followed by washing with conductivity water till the wash liquid showed no visible turbidity with an acidified solution of silver nitrate. In later work acid clays prepared by the hydrochloric acid treatment were electrodialysed to ensure removal of any free hydrochloric acid from the sol.

The following table gives the source and the method of preparation of the different clay acids.

Sol	Source	Method of preparation
A . .	Silt from Ganges water (sediments at the bottom of storage tanks containing Ganges water).	Suspension obtained bycentrifuging only not followed by any acid treatment or electrodialysis.
A_1 . .	Ditto . .	Acid treatment followed by leaching with water to remove any free hydrochloric acid.
B . .	Dacca (Bengal) virgin soil (collected from a depth of 0″—6″).	Ditto.
B_1 . .	Ditto . .	Acid treatment followed by leaching with water and then electrodialysis.
C . .	Dacca (Bengal) cultivated soil (unmanured crop ; collected from a depth of 0″—6″).	Electrodyalisis only.
C_1 . .	Ditto . .	Acid treatment followed by leaching with water and then electrodialysis.

The sol was in all cases stocked in an atmosphere of hydrogen and the arrangement as described in a previous paper [Mukherjee *et al.*, 1932] was used for this purpose. The titration cell for simultaneous conductometric and potentiometric measurements was also as previously used [Mukherjee *et al.*, 1932] with the modification that ground-in mercury seal stirrers were employed to ensure a rapid and thorough intermixture between the sol and the titrant liquid. A Leeds and Northrup potentiometer reading directly to 0·1 millivolt and a galvanometer with a current sensitivity of 10^{-8} ampere were used for potentiometric measurements with sols A and A_1. With the remaining sols a Leeds and Northrup K-type potentiometer and a galvanometer with a current sensitivity of 10^{-11} ampere were employed. Electrolytic hydrogen purified as already described [Mukherjee *et al.*, 1932] was used. All measurements were carried out in an electrically heated water thermostat maintained at a temperature of 35°C. $\pm$ 0·05. In view of the high resistances of the systems special precautions had to be taken to guard against electrical leakages which are particularly troublesome during the rainy season [Mukherjee *et al.*, 1936.]

Reproducibility of results

In connection with previous work with silicic acid sols it was found that the pH values of the sols sometimes showed marked variations. The clay acids appear to behave in an analogous manner in this respect though the variations are not so wide as the following Table I will show. The pH obtained from the hydro-

gen electrode has, as in previous work, been often checked with the quinhydrone electrode. Inspite of appreciable initial differences the titration curves of a given sol with the same alkali run almost parallel and are generally identical in form. The initial differences arising, probably from a poisoning of the electrode, do not affect the total acidity values calculated from the curves. The point is illustrated, among others, by curves 3 and 4 for sol A_1, and curves 16 and 17 for sol C.

Table I shows the variations in the pH and the specific conductivities of the different sols. The observed e. m. f. against the normal calomel electrode is given in the first and second columns; the e. m. f. of the experimental cell against the normal hydrogen electrode is given in the third and fourth columns, the e. m. f. of the normal calomel half element being taken to be 0·2823 volt at 35° C. The maximum difference from the average e. m. f. is given in percentage of the average value in the fifth column; the sixth and seventh columns give the pH calculated from the hydrogen and quinhydrone electrode separately. The specific conductivities are given in the last column.

TABLE I

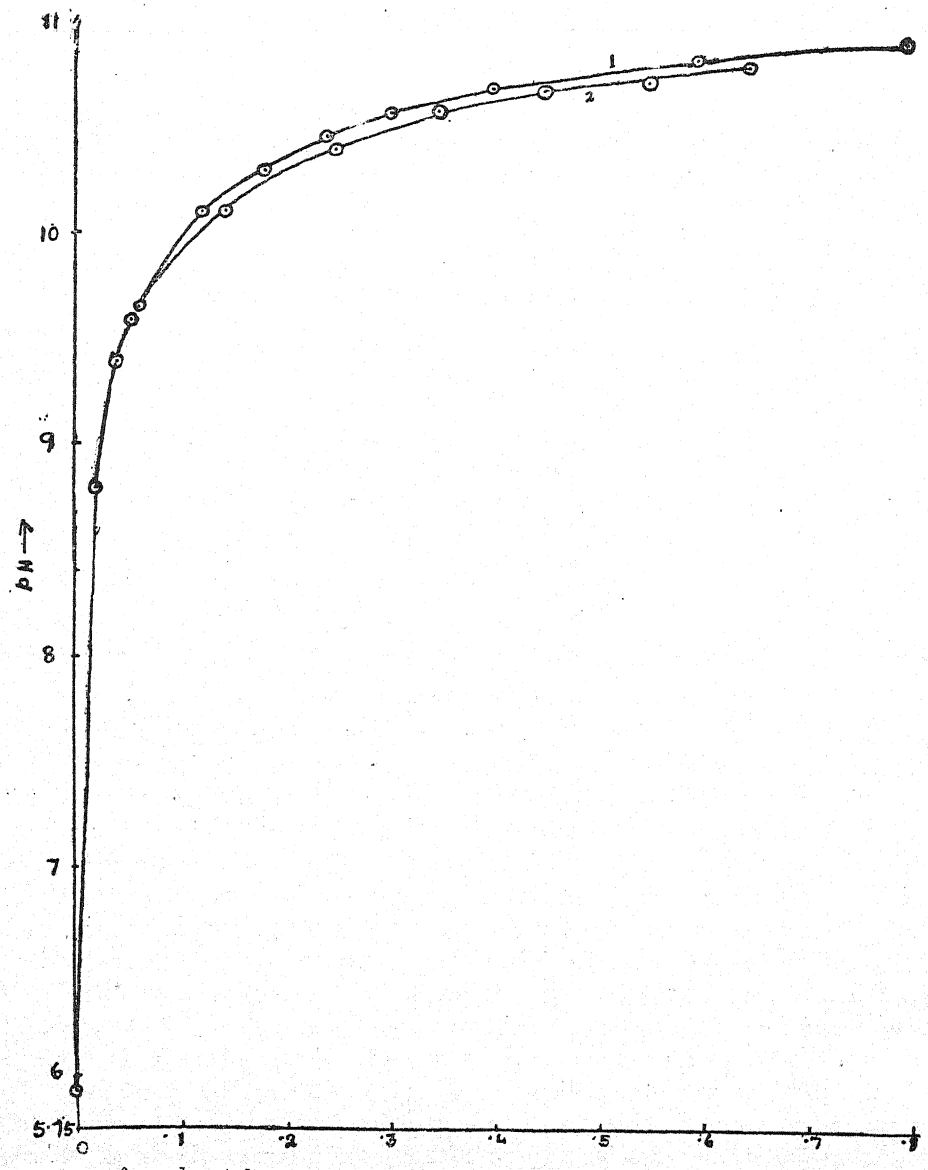
	E_{obs}	E_{obs}	E_h	E_h	Maximum difference from average e. m. f. with hydrogen electrode (in percentage)	pH		Sp. conductivity in mhos
	Hydrogen electrode in volts	Quinhydrone electrode in volts	Hydrogen electrode in volts	Quinhydrone electrode in volts		Hydrogen electrode	Quinhydrone electrode	
Sol A—								
	0·6450		0·3627			5·93		
	0·6480		0·3657			5·98		
	0·6530		0·3707			6·07		
	0·6550		0·3727			6·10		
			average			average		
			0·3680		—1·4	6·02		
					+1·1			
Sol A_1 —								
	0·5500		0·2677			4·38		
	0·5530		0·2707			4·43		
	0·5480		0·2657			4·35		
	0·5460		0·2637			4·31		
	0·5450		0·2627			4·30		
			average :			average		
			0·2661		—1·3	4·35		
					+1·3			
Sol B—								
	0·6080		0·3257			5·33		
	0·6140		0·3317			5·43		
	0·5900		0·3077			5·03		
		0·0890		0·3203				$5·24 \times 10^{-5}$
			average			average		
			0·3235		—4·9	5·29		
					+2·5			
S B_1 —								
	0·5850		0·3027			4·95		
	0·5805		0·2982			4·88		
	0·5830		0·3007			4·95		
	0·5902		0·3097			5·04		
	0·5800		0·3077			4·87		
	0·5785		0·2962			4·85		
			average			average		
			0·3006		—1·4	4·92		
					+2·4			

TABLE I—*contd.*

	E_{obs}	E_{obs}	E_h	E_h		pH		
	Hydrogen electrode	Quinhydrone electrode	Hydrogen electrode	Quinhydrone electrode	Maximum difference from average e. m. f. with hydrogen electrode (in percentage)	Hydrogen electrode	Quinhydrone electrode	Sp. conductivity in mhos
Sol C—								
	0·4935		0·2112			3·46		
	0·5050		0·2227			3·64		
	0·5070		0·2247			3·67		$5·75\times10^{-5}$
	0·4955		0·2132			3·49		$5·24\times10^{-5}$
	0·5090		0·2267			3·71		$5·42\times10^{-5}$
	0·4920		0·2097			3·43		
		0·1900		0·2193			3·59	
		0·1895		0·2198			3·60	
			average			average		
			0·2180		—3·9	3·58		
					+4·0			
Sol 1—								
	0·5880		0·3057			5·00		
	0·5963		0·3142			5·14		$2·09\times10^{-5}$
	0·5975		0·3152			5·16		$2·21\times10^{-5}$
	0·5817		0·2994			4·90		$2·15\times10^{-5}$
		0·1038		0·3055			5·00	
			average			average		
			0·3086		—0·9	5·05		
					+1·1			

GANGES SILT

Sediments collected from the bottom of storage tanks containing Ganges water were air-dried, powdered, and sieved as usual, and after removal of organic materials with six per cent hydrogen peroxide the colloidal portion was separated with the aid of a Sharple's Supercentrifuge. As it was intended to investigate, in the first instance, the acid properties, if any, of the sediments as they occur in nature neither any preliminary acid treatment nor electrodialysis was taken recourse to in the preparation of the sol (sol A). Curves 1 and 2 (Fig. 1) represent the results of potentiometric titration of this sol with alkalis. The sol shows a practically neutral reaction and the curves are characteristic of very weak acids ; the flat portions of the curves correspond with the region where the sol coagulated. In contrast however, with a true acid character the final ascending portion is absent. The flat portions appear to indicate an adsorption of the alkali by the coagulum in agreement with the observations of previous investigators [Bradfield, 1924] regarding the strong adsorbing power of soil colloids for alkalis in the alkaline region.



c. c. of moderately strong Ba $(OH)_2$ (0·297*N*) added to 25 c. c. of sol.

Fig. 1. Sol A, Ganges silt.

On account of the low acid reaction of the above sol a portion was coagulated with 0·05 *N* hydrochloric acid and the coagulum leached several times with this acid ; the residue was then washed on an ultrafilter with conductivity water till the ultrafiltrate showed no visible turbidity with an acidified solution of silver nitrate. On now vigorously shaking the residue with conductivity water a concentrated suspension was obtained which on centrifuging gave a fine stable suspension (sol A_1).

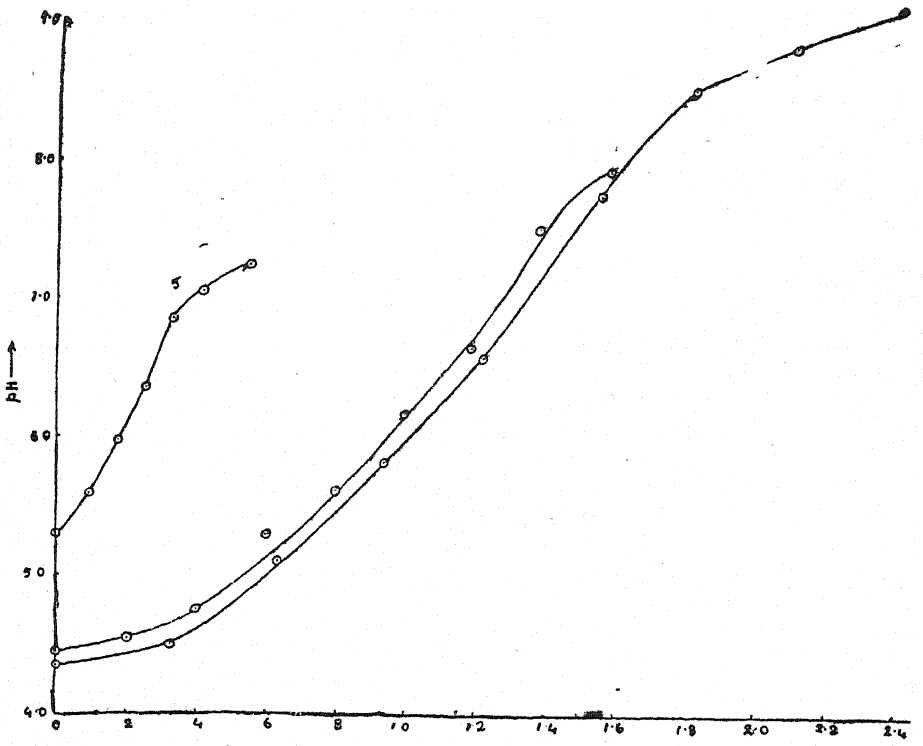
The titration curves 3 and 4 were obtained with dilute alkali. The results are summarised in the following table :

TABLE II

	Total acid $\times 10^5$ in normality	pH (average)	Free acid in normality	Free acid / Total acid	Cl-ion concentration $\times 10^5$
Sol A_1 . .	33·2 (curve 3) . 31·1 (curve 4) .	4·35	$4·5 \times 10^{-5}$	·14	5·00
Ultrafiltrate of Sol A_1 . .	..	5·4	$4·0 \times 10^{-6}$	..	5·00
Sol $A_1/4$ . .	7·5 (curve 5) .				

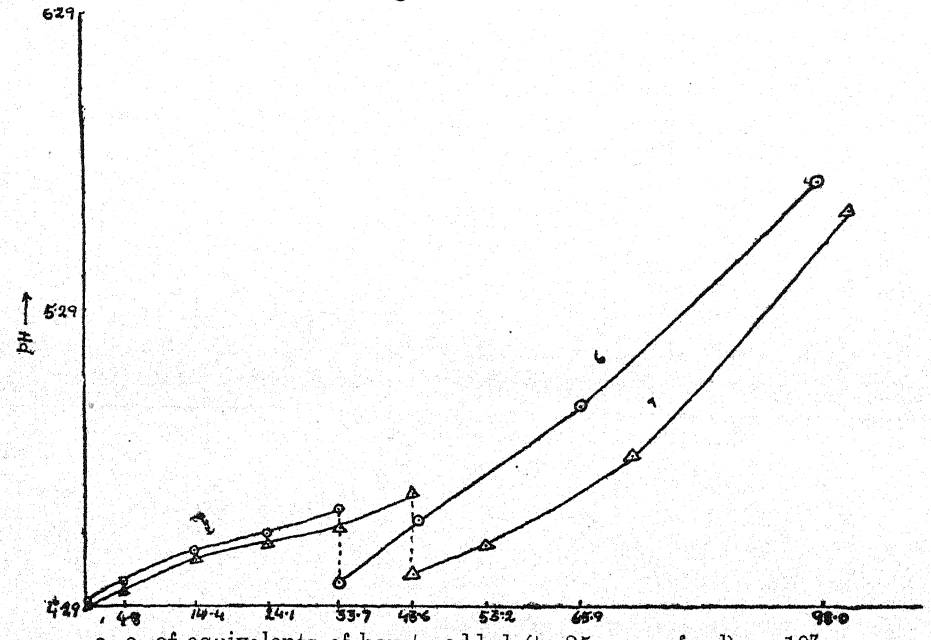
The titration curves 3 and 4 (Fig. 2) indicate a strong acid character. The curves are fairly smooth,—the breaks indicating a polybasic character observed by some previous investigators are not in evidence. The chlorine-ion concentration of the sol calculated from the observed e. m. f. of the cell Ag-AgCl/·01 *N* HCl/sat. KNO_3/sol/AgCl-Ag is the same as that of the ultrafiltrate. In view of the weak acid reaction of the ultrafiltrate and the small chlorine-ion concentrations it is not possible to refer the observed acid character of the sol to the presence of free acids in the intermicellar liquid. The strong acid character disappears on titrating with concentrated alkalis (curve 8 ; Fig. 4) ; the particles coagulate and the strong buffer action in the region corresponding with the flat portion of the curve indicates a surface reaction involving the disappearance of considerable amounts of the alkali*. The clay sols, in this respect, appear to behave in an analogous manner with silicic acid sols [Mukherjee *et al.*, 1936] with the difference that with concentrated alkalis the clay particles coagulate instead of dissolving.

* More detailed investigations in this direction are in progress.



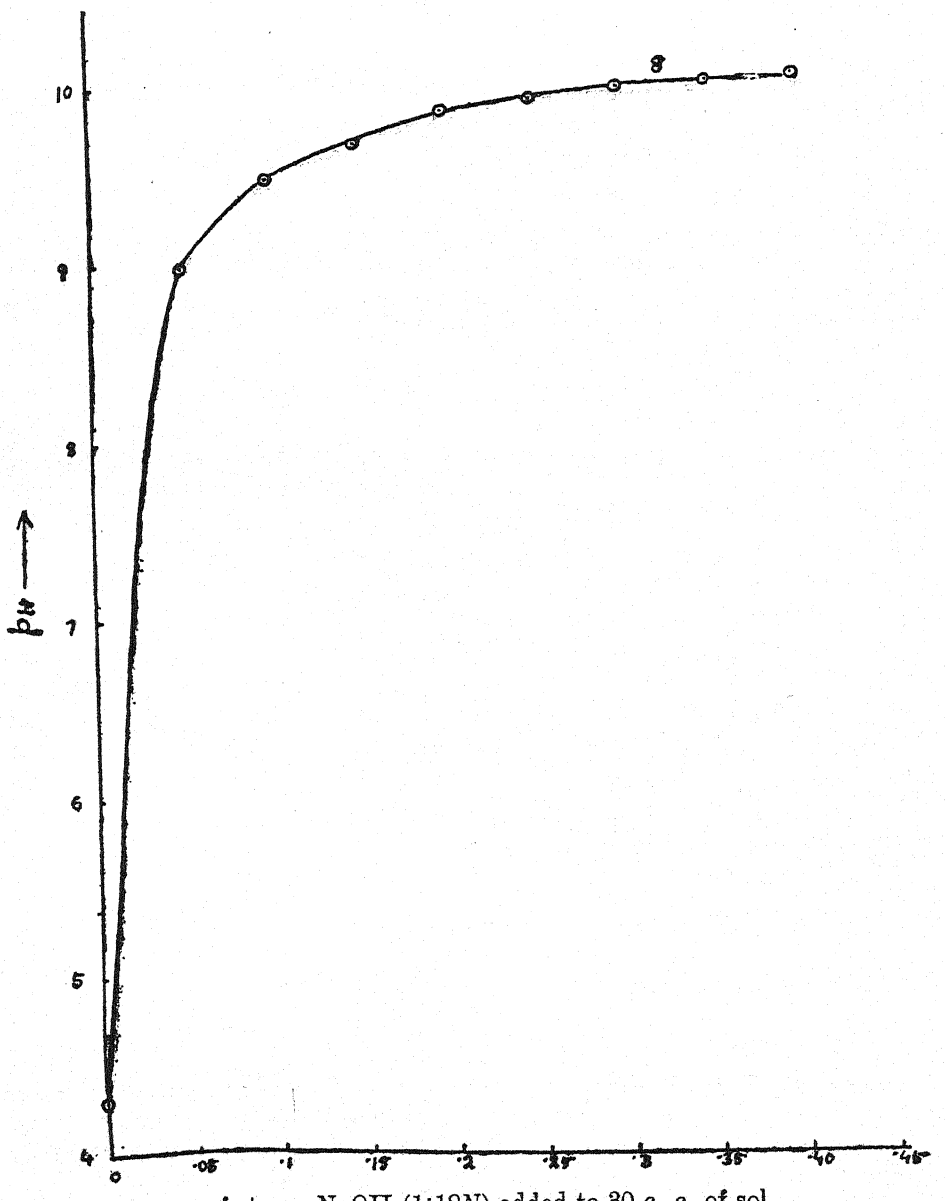
c. c. of 0·0063 *N* baryta added to 25 c. c. of sol.

Fig. 2. Sol A.



c. c. of equivalents of baryta added (to 25 c. c. of sol) × 10^7.

Fig. 3. Sol A (Ganges sediment).



c. c. of strong NaOH (1·18*N*) added to 30 c. c. of sol.

Fig. 4. Sol A.

The low ratio of free to total acid of the sol indicates a weak acid character. The accuracy of the absolute value of this ratio is somewhat ill defined in view of uncertainties of absolute measurements with the hydrogen electrode with unbuffered solutions of extremely low hydrogen-ion concentration, especially in the absence of independent measurements with the quinhydrone electrode. Further, the error of the e. m. f. determination may be multiplied several times in calculating the hydrogen-ion concentrations from the pH values as it depends on the magnitude of the mantissa in converting it into its antilog.

In the titration of the acid clays with alkalis the time allowed for interaction has been found to be a very important factor. It has been repeatedly observed that on the addition of a given amount of alkali after the electrode has been in equilibrium with the sol to be titrated, the e. m. f. of the hydrogen electrode at first rises to a maximum and then diminishes very slowly till equilibrium is reached. This behaviour is observed near about the neutral region and it becomes pronounced in the alkaline region ; a slow adsorption of the alkali by the particles probably takes place ; the need of vigorous shaking during the titration is obvious.

The effect of the above slow reaction between the acid clay suspension and the alkali in which the alkali appears to disappear becomes more pronounced when a titration is continued for several days. It has been repeatedly observed that the initial pH on any day is lower than the final equilibrium pH (after the titration had been carried to a definite point) on the day previous to it. Curves 6 and 7 (Fig. 3) illustrate this point with Sol A_1. This observation is in contrast with our experience with silicic acid sols where, under similar circumstances, a slight increase in the initial pH on successive days has been observed. The effect of the above slow reaction has to be considered in the interpretation of the results of a titration where a second addition of the alkali was made when the e. m. f. of the hydrogen electrode corresponding to the previous addition did not change in half an hour's time.

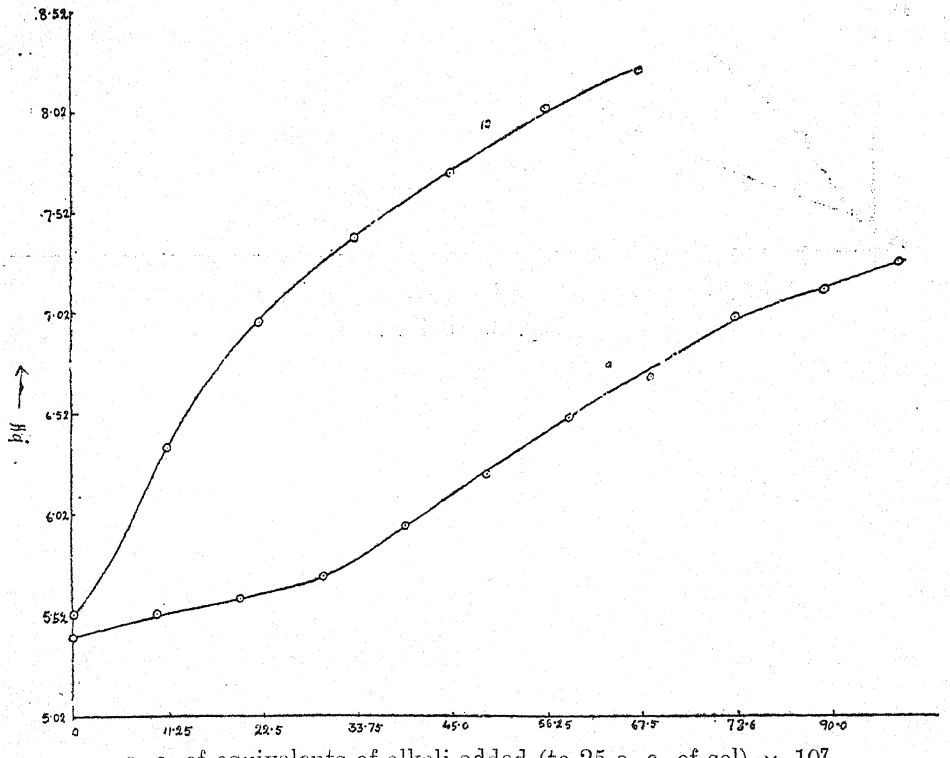
Sols B and B_1

The results with these sols are summarised below :

Table III

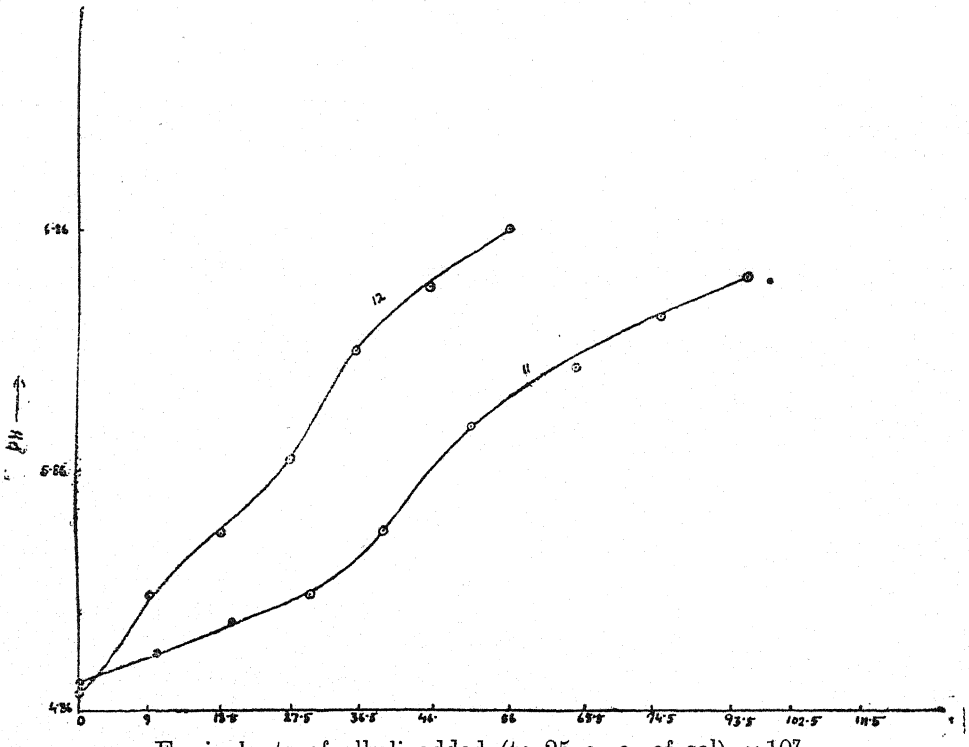
	Total acid × 10^5 in normality	pH (Average)	Free acid in normality
Sol B	18·0 (With $Ba(OH)_2$; curve 9) 7·0 (With NaOH ; curve 10)	5·29	$6·0 \times 10^{-6}$
Sol B_1	15·0 (With $Ba(OH)_2$; curve 11) 6·6 (With NaOH ; curve 12)	4·92	$1·2 \times 10^{-5}$

The titration curves with dilute alkalis resemble those of moderately strong acids ; with concentrated alkalis a weak acid character is indicated (compare sol A_1), the particles coagulating at the higher alkaline regions. The titration curve with barium hydroxide is different from that with caustic soda—a strong buffer action being observed with barium hydroxide. This observation is in agreement with the results of previous investigators with clay acids. Unpublished work from this laboratory with palmitic acid sols also point to a difference in total acidity on titration with different strong bases. These results support Mukherjee's previous conclusion based on theoretical considerations regarding the indefiniteness of the total amount of the neutralisable acid in colloidal acid systems [Mukherjee *et al.*, 1934]. As pointed out by Mukherjee the characterisation of the intensity factor of such acids in terms of the mass action expression becomes ambiguous because of the above indefiniteness of the total acid.



c. c. of equivalents of alkali added (to 25 c. c. of sol) $\times 10^7$.

Fig. 5. Sol B.



Equivalents of alkali added (to 25 c. c. of sol) $\times 10^7$.

Fig. 6. Sol B.

Sol C

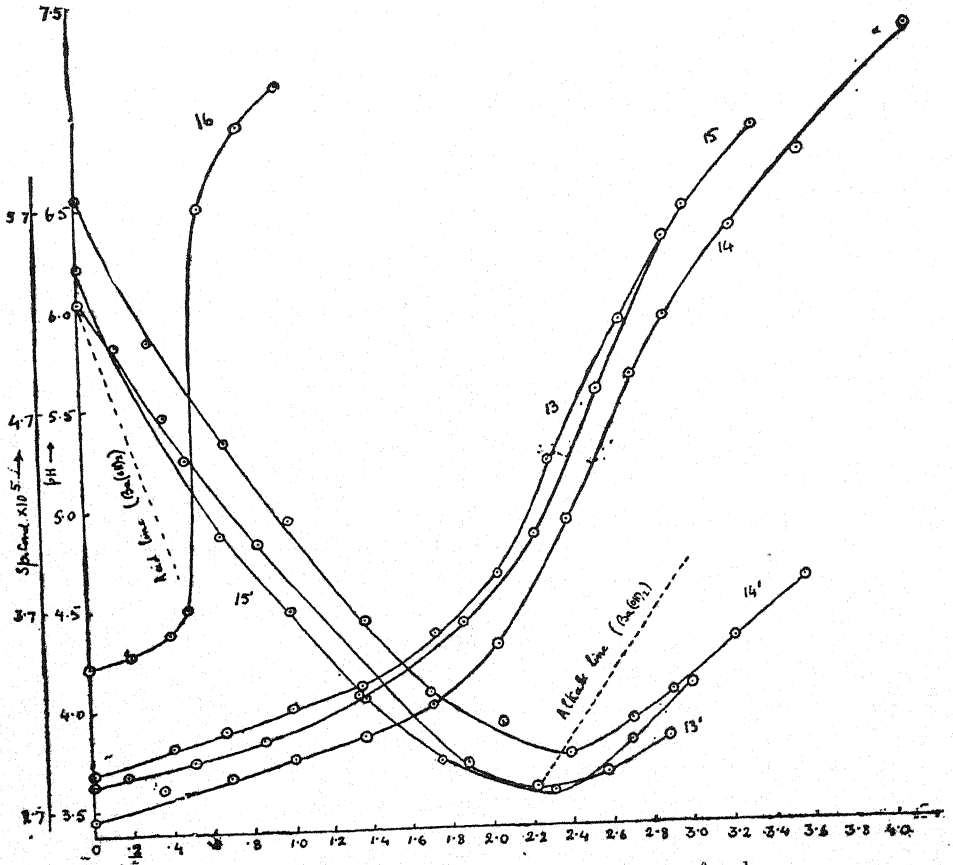
In the preparation of sols A_1, B and B_1 a preliminary leaching with hydrochloric acid had been taken recourse to. The acid character of the sols might be attributed to hydrochloric acid adsorbed by the particles. It was therefore considered desirable to prepare an acid clay sol by electrodialysis alone omitting the hydrochloric acid treatment. Sol C was prepared in this manner after removal of organic matter by the usual treatment with six per cent hydrogen peroxide. The electrodialysis was effected in glass vessels with parchment membranes and platinum electrodes. The results are given in Table IV.

TABLE IV

	Total acid $\times 10^4$ in normality	pH (average)	Free acid $\times 10^4$ in normality	$\frac{\text{Free acid}}{\text{Total acid}}$	Chlorine-ion conc. $\times 10^5$ in normality
Sol C . .	2·8 (curves 13, 14, 15 & 13′, 14′, 15′)	3·58	2·8	1·0	9·4
Ultrafiltrate of Sol C	0·7 (curve 16) .	..	..	..	9·5

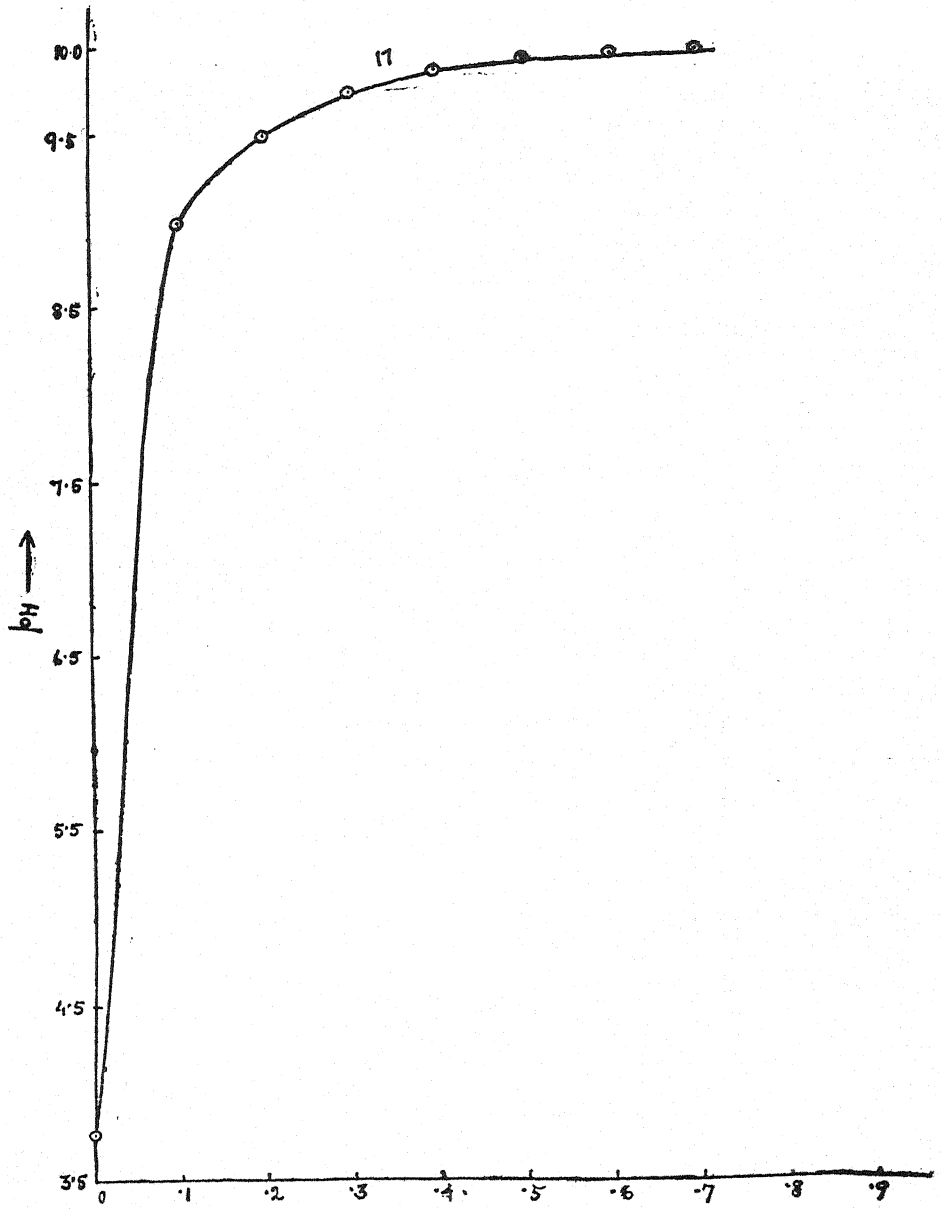
The sol is distinctly more acid than any of the previous sols. The titration curves 13, 14, 15 and 13′, 14′, 15′ (Fig. 7) indicate a fairly strong acid character which is confirmed by the agreement between the free and total acids. The titration curve 17 (Fig. 8) with strong alkali indicates however, a weak acid character, though the final rise after a flat portion is not in evidence. The total acids calculated from the potentiometric and conductometric titration curves are identical and in contrast with the previous sols is the same with sodium and barium hydroxides. The conductometric titration curves on closer examination suggest a slow reaction with the alkali near the point of neutralisation. A comparison of the slope of the experimental curve with that of the theoretical alkali line indicates that the above slow reaction, involving probably an adsorption of the hydroxyl ions by the colloidal particles, is continued even after the minimum point has been passed. The acid clay sols show a striking similarity with colloidal solutions of silicic acid in this regard. The chlorine-ion concentration of the sol calculated from the e. m. f. of the cell Ag-AgCl/0·01 *N* HCl/Sol KNO_3/Sol/AgCl-Ag agrees with that of the ultrafiltrate obtained in an analogous manner. The small chlorine-ion concentration shows that the large free and total acids of the sol cannot be wholly attributed to free hydrochloric acid. The total acid of the ultrafiltrate is about twenty-five per cent of the total acid of the sol. The acidity of the ultrafiltrate is probably due to free hydrochloric acid as is shown by the

approximate agreement between the total acid and the chlorine-ion concentration of the ultrafiltrate. Further, the potentiometric titration curve 16 of the ultrafiltrate is characteristic of a very strong acid. As the preliminary hydrochloric acid treatment was omitted in the preparation of the sol it is interesting to find the presence of free hydrochloric acid in the sol.



c. c. of 0·0064*N* alkali added to 50 c. c. of sol.

Fig. 7. Sol. C.



c. c. of strong NaOH (0·343*N*) added to 40 c. c. of sol.

Fig. 8. Sol C.

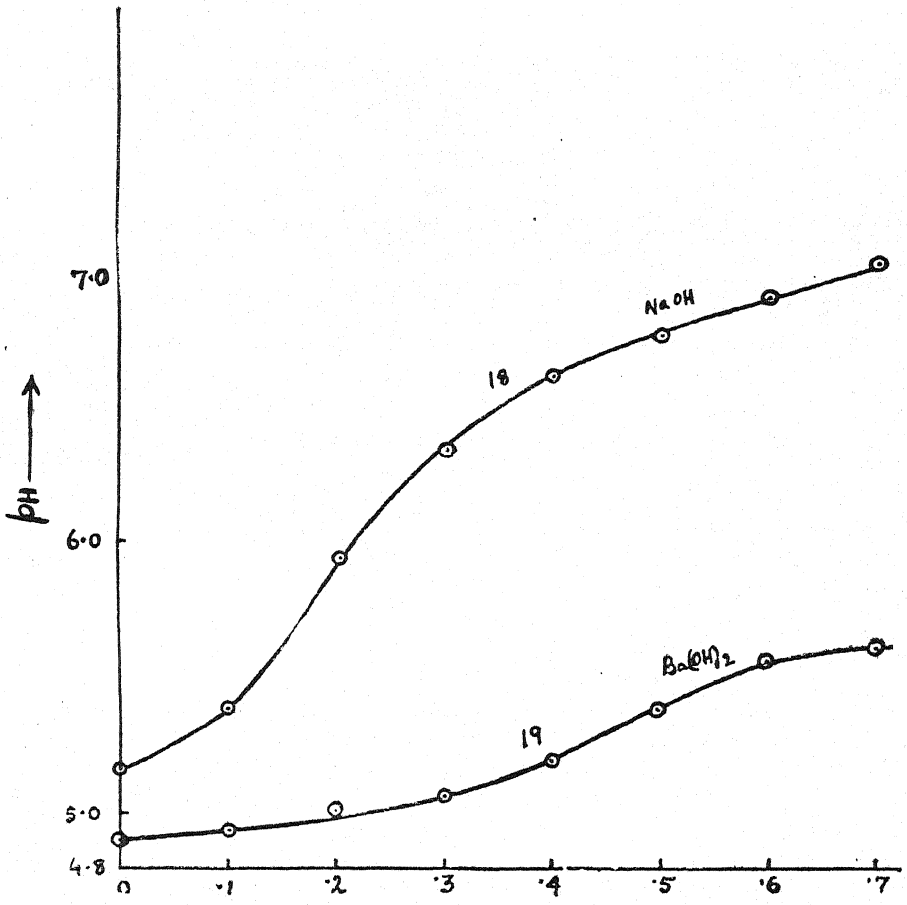
Sol C_1

This sol was isolated from the same soil sample as sol C but in preparing it the electrodialysis was preceded by a preliminary leaching of the colloidal material with 0·05 *N* hydrochloric acid. The results are given in Table V.

TABLE V

	Total acid	pH (average)	Chlorine-ion conc.
Sol C_1 . . .	$1·91 \times 10^{-5}$ *N* (with NaOH ; curve 18). $5·7 \times 10^{-5}$ *N* (with $Ba(OH)_2$; curve 19).	5·05	*Nil*
Ultrafiltrate of sol C_1	..	6·4	*Nil*

The acid character of clay acid appears to depend, among other factors, on the method of preparation of the sol. The difference in the electrochemical behaviours of sols C and C_1 isolated from the same soil sample illustrates this point. Sol C is definitely more acid than sol C_1. Unlike sol C the titration curves 18 and 19 (Fig. 9) of sol C_1 with sodium and barium hydroxides show a difference ; also the total acid is greater than the free acid. Sol C is a dilute sol containing 0·44 grm. of the colloid per litre while the colloid content of sol C_1 is 0·72 grm. per litre. Also while sol C is practically free from iron, sol C_1 contains eighteen per cent ferric oxide in terms of the oven-dried residue. This difference in ferric oxide content probably arises from the fact that whereas sol C_1 had been electrodialysed after a preliminary leaching with dilute hydrochloric acid sol C was electrodialysed directly. The above difference in composition of the colloidal material is probably responsible for the incongruous features presented by the two sols.



c. c. of 0·0064*N* alkali in 50 c. c. of sol.

Fig. 9. Acid clay sol C_1 .

Neutral salts liberate (see later) considerable amounts of acid with both the sols. In view of the practically complete agreement between the free and total acidities of sol C, it is most interesting to note the liberation of acids by neutral salts from this sol. This observation is of fundamental importance in relation to the theoretical treatment of the properties of colloidal acids.

Liberation of acids by salt solutions

Titrations of colloidal clay acids by salt solutions are of interest as throwing light, among other things, on the mechanism of the phenomena of exchange acidity of soils. Systematic investigations are necessary to understand the exact nature of the reactions involved. The results of experiments in this direction are given below:

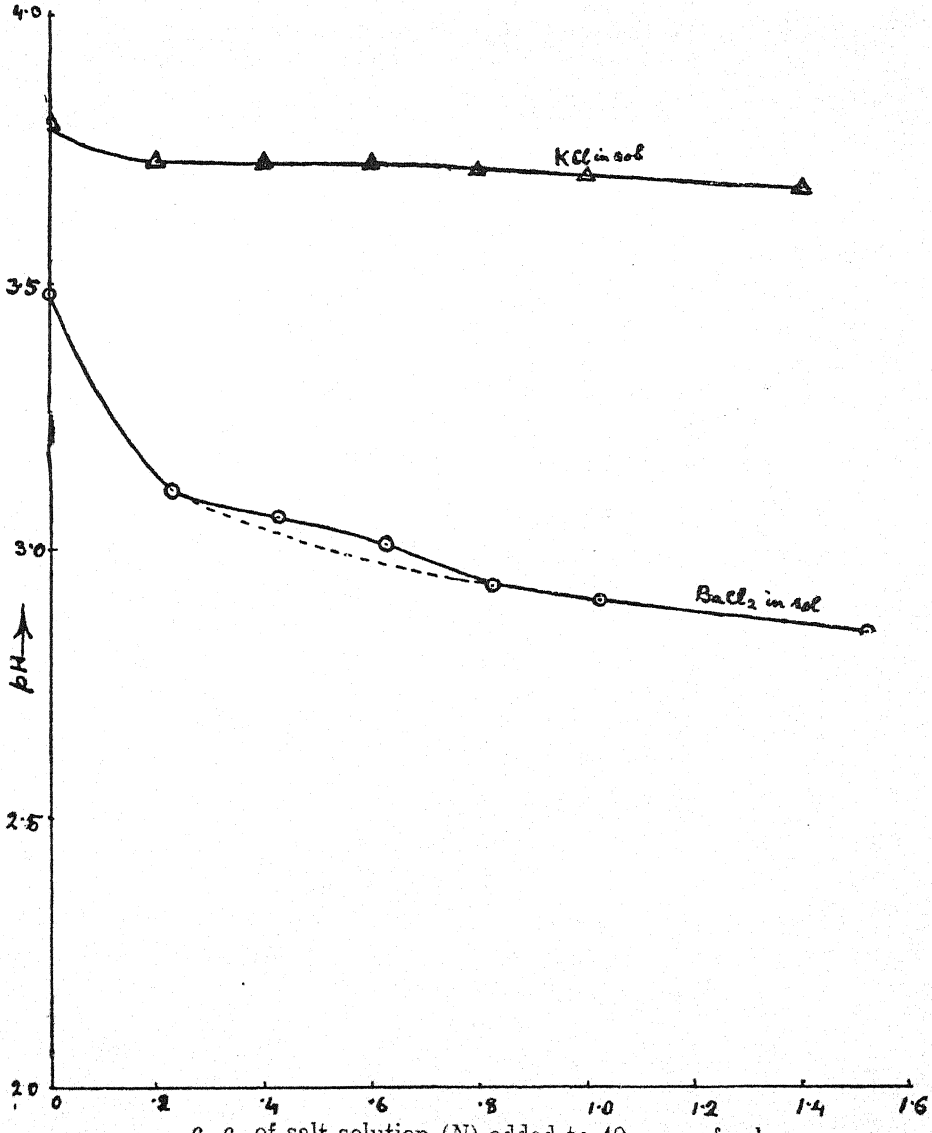
	Total acid obtained by titration with alkalis without addition of salt	Initial pH (without salt)	Conc. of salt added	pH after addition of salt	Curve number
Sol C .	$2 \cdot 8 \times 10^{-4}$ *N* .	3·48	0·037*N* $BaCl_2$.	2·85	20
	Do. .	3·80	0·034*N* KCl .	3·67	21
Sol C_1 .	$1 \cdot 91 \times 10^{-5}$ *N* (with NaOH)	5·13	0·006*N* $BaCl_2$.	4·58	22
	$5 \cdot 7 \times 10^{-5}$ *N* (with $Ba(OH)_2$).	5·05	0.006*N* KCl .	4·85	23

It will be seen that neutral salts liberate considerable quantities of acids from the clay acids—barium chloride being more powerful than potassium chloride in this respect [Mukherjee *et al.*, 1936]. As already stated the liberation of acids in the case of sol C is particularly interesting in view of the apparent strong acid character of the sol as indicated by the form of its titration curves as also by the agreement between the free and total acids.

According to Mukherjee [1922] the liberation of acids by neutral salts arises from an interchange between the cation of the added electrolyte and the hydrogen-ions electrically adsorbed on the surface of the colloidal particles. Exchanges with such cations will not alter the observed hydrogen-ion concentration of the medium so long as only the mobile hydrogen ions interchange their places with the cations in the bulk of the solution. The osmotic penetration of the cations into the double layer may, under favourable circumstances, produce a shift in the distribution of the hydrogen ions between the primarily adsorbed layer and the mobile layer, and thus produce a variation in the observed hydrogen-ion activity. The interchange is governed by the relative adsorbabilities of the cations depending on their valencies, mobilities and states of hydration. One thus obtains the well-known valency series when electric adsorption alone determines the adsorbability. It is interesting to find that in all the above cases the concentration of hydrogen-ions in displacement equilibrium with different salts is in agreement with

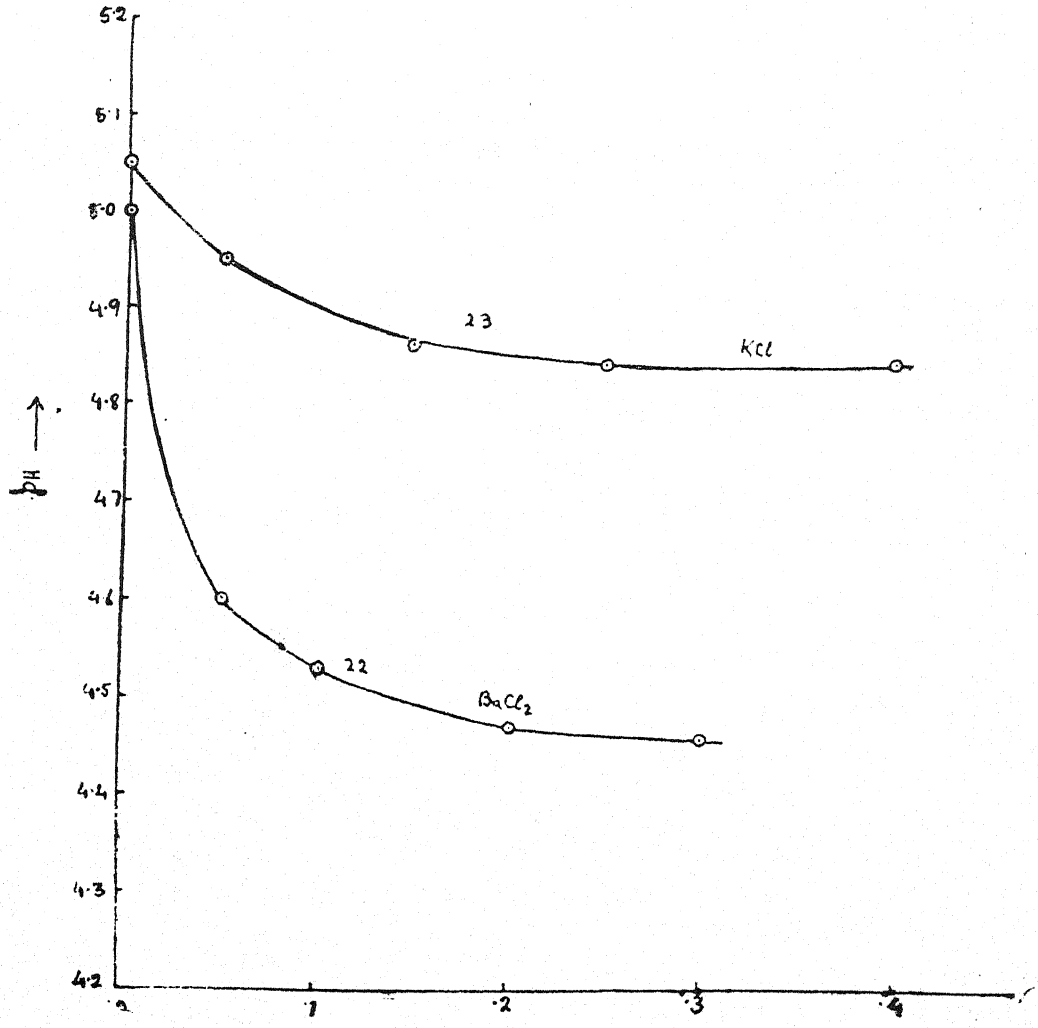
the above series. Barium chloride in all cases liberates more acid than the alkali metal salts.

The liberation of acids by neutral salts in the case of colloidal acids which are apparently completely dissociated (*e.g.* acid clay sol C) as is shown by the equality of their free and total acids is difficult to understand in the light of classical electrochemistry as such acid liberation is tantamount to an incomplete dissociation.



c. c. of salt solution (N) added to 40 c. c. of sol.

Fig. 10. Acid clay sol C.



c. c. of *N* salt solution added to 40 c. c. of soi.

FIG. 11. Sol C.

Basicity of acid clay sols

Colloidal clay acids have often been looked upon as polybasic acids in true solution. The titration curves given in this paper bear no evidence of the different stages of dissociation characteristic of polybasic acids and are typical of monobasic acids. The alkaline regions of the curve also resemble those of monobasic acids and the adsorption of alkalis by the colloidal particles is sufficient to account for their form. In agreement with the observation of some previous investigators regarding stages of dissociation of soil acids sudden changes in the slopes of the titration curves have sometimes been observed in this laboratory but they disappeared when the colloidal solution was stirred vigorously during the titration. These sudden changes simulate the stages of dissociation of a polybasic acid and arise out of local increases in the concentration of the alkali which has not mixed thoroughly with the whole liquid.

The intensity factor of soil acidity

While several methods exist for determining the total acidity and the degree of saturation of soils, it has not yet been possible to obtain an estimate of the intensity factor of the acidity. If the clay acids could be treated as acids in true solution, titration curves would afford useful information regarding the total acidity and the intensity factor expressed as the dissociation constant. The foregoing results and also previous work from this laboratory show that the total amount of acid taking part in the interaction with alkalis is a variable quantity depending, among other things, on the nature and concentration of the alkali and on the extent of dispersion of the acid substance and is not simply determined by its total concentration. The interaction involving the interface constitutes the difference.

The conception of a secondary ion adsorption (electrical and chemical adsorption) put forth by Mukherjee offers a better understanding of the characteristic behaviour of colloidal acids. According to this view a colloidal acid clay particle owes its charge (negative) to the presence on its surface (by primary adsorption) of a fixed layer of ions. Corresponding to the number of these ions an equivalent number of oppositely charged ions remain in the liquid near the surface. A layer of oppositely charged ions may remain fixed on the surface by electrostatic and valence forces thus forming an osmotically inactive secondarily adsorbed layer on the surface. As already stated the liberation of acids by neutral salts is, on the above view, brought about by the interchange between electrically adsorbed hydrogen ions and the cations of the added electrolyte. The difference in the forms of the titration curves with sodium and barium hydroxides results from a stronger adsorption of the barium ion. The buffer action, particularly with barium hydroxide, in the acid region indicates the possibility that the surface molecules of the acid which are 'built into' the crystal lattice of the particles may have a stronger acid character than those in true solution.

According to the above view the nature of the primarily adsorbed ions and their state of adsorption determine the 'strengths' of colloidal acids. Experimental methods throwing light on the mechanism of interchanges between the ions in the 'double layer' and the ions in the intermicellar liquid can be outlined. Thus the necessary information regarding the condition and the nature of the primarily adsorbed ions may be obtained. These aspects of the problem will be later more fully discussed.

Summary

1. The electrochemical behaviours of several acid clay sols isolated from a number of soil samples with the aid of Sharple's Supercentrifuge have been investigated. The intensity of the acid character of these sols have been discussed with reference to the nature of their titration curves with different strong bases and with different concentration of alkalis.

2. The initial pH values calculated from the e. m. f.'s of the hydrogen electrode sometimes show wide variations due, probably, to a poisoning of the hydrogen electrode. The form of the titration curve is, however, very little affected by a 'poisoned' electrode and the total acidity of a given sol calculated from different curves show satisfactory agreement inspite of appreciable initial differences of the curves.

3. Titration curves with dilute alkalis point to a moderately strong acid character. With concentrated alkalis a weak acid character is indicated. The curves are remarkably smooth. No evidence of stages of dissociation are obtained.

4. The titration curves give evidence of reactions distinct from a simple neutralisation of hydrogen by hydroxyl ions. The curvature of the conductometric titration curves in the acid region and near about the endpoint, point to an adsorption of the alkali by the interface.

5. The total acid calculated from the titration curves is different with different strong bases. With concentrated alkalis the particles coagulate in the alkaline region and the titration curves simulate those of weak acids with the difference that the final rise after a flat portion is not observed even when the alkali added would neutralise several times the total acid calculated from the titration curves with dilute alkalis. The total acid is thus an indefinite quantity.

6. Neutral salts liberate considerable amounts of acids from the sols—the alkaline earth cations being more effective in this respect than the alkali metal cations. The effect of addition of neutral salts has been discussed from the point of view of electrical adsorption of ions.

7. The results indicate certain characteristic behaviours of the clay acids and en-phasise the need of a theoretical treatment of these acids which must differ from that of acids in true solution.

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INDIAN SOIL FUNGI

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In the earlier days of soil microbiology, attention was focussed almost exclusively on the bacteria present. It is now realised that saprophytic moulds and other fungi are also of great importance in soil processes. They are, for example, the principal agents in the decomposition of cellulose and hemicelluloses of dead plant material, and are capable of carrying out their activities with lower moisture requirements than are the bacteria. Certain soil fungi as well as soil bacteria are capable of producing ammonia from the protein of plant and animal debris, thus assisting to make the organic nitrogen once more available for plant growth, although the later stages of conversion are left to the nitrite- and nitrate-forming bacteria. Conversely, nitrates and ammonium salts in the soil are converted by soil fungi into organic nitrogen, "locked up" in the fungal tissues, and only available to crop plants after the breakdown of those tissues.

Another important aspect of soil saprophytes is the part they play in suppressing potential plant pathogens present in the soil. This may occur simply because they are better adapted to a saprophytic life and are thus able to starve out the parasitic fungi ; or, as has been shown for certain instances [Weindling, 1932], the saprophyte may produce a definite toxin.

A complete study of the fungus flora of soil presents many difficulties. Not all the organisms present are capable of growing on artificial media, and many of the pathogenic fungi can only be detected by their effect on crop plants. The fungi that develop on laboratory media are the saprophytes and facultative saprophytes, the majority of which belong to the difficult group of Fungi Imperfecti. Identification is a matter of considerable difficulty, and no "host index" can be consulted as in the case of a plant parasite.

Owing to the fact that fungi exist in the soil both as mycelium and spores, the numbers of colonies obtained on dilution plates of a soil sample by no means indicate the relative importance of these organisms in the soil. A cluster of dormant spores will produce many colonies, whereas a mass of active mycelium may give only one.

Bacteria in the soil so out-number the fungi that precautions have to be taken to suppress bacterial growth—if possible without affecting fungal growth. A fairly close approach to this can be made by slightly acidifying the medium to a degree that checks bacteria but that has little effect on the majority of soil fungi.

Subject to these limitations, the "plating out" technique gives a useful indication of the types of saprophytic fungi present. It may be supplemented by "direct examination" of mycelium in the soil, and by "direct isolation" of the mycelium that is first put out from particles of soil placed on an agar medium and incubated. Surveys of soil fungi have been carried out in various countries, and the most striking point brought out is the universal distribution of the commoner soil organisms. This is a point of economic interest, for most "air infections" are really soil organisms accompanying dust particles; hence the moulds causing decay of foodstuffs, textiles, and similar industrial and agricultural products show a remarkable similarity all over the world, and uniform methods of control can be adopted. The work of Dale [1912, 1914], and Brierley [1923] in England; of Jensen [1912], Waksman [1916, 1917], Werkenthin [1916], Gilman and Abbott [1927], and LeClerg [1931] in America; Bisby and his co-workers [1933, 1935] in Canada; Oudemans and Koning [1902] in Holland, and Jensen [1931] in Denmark, may be mentioned, and a good summary of the earlier work is given by Waksman [1932].

In India, little attention has been given to soil fungi. It is very desirable that the soil flora of this country should be more extensively studied; as, however, it is not clear that such a study would lead to immediate practical results, this work is perhaps more suitable to the Universities than to research workers of the agricultural departments. Shaw [1915] records four isolations from Pusa soils; Thakur and Norris [1928] describe about twenty-five species from Madras soil, with special reference to their power of cellulose decomposition and ammonification; Mason [1928] mentions five species isolated by Rhind in Burma; and finally Chaudhuri and Sachar [1934] give full descriptions of thirty-two species isolated from soil samples taken at Lahore.

TABLE I

Soil samples for isolation of fungi

No.	Source	Date	Moisture per cent	pH
	Alkaline soils			
1	Pusa Garden soil	Feburary .	6·5	8·5
2	,, ,, ,, . . .	September .	16·0	7·9
3	,, Experimental plot . .	March . .	4·5	8·0
4	,, ,, ,, . .	September .	15·0	8·3
5	,, Kitchen garden . . .	March . .	13·8	8·5
6	,, ,, ,, . . .	October .	13·7	8·05
7	,, Low land by river . .	March . .	18·3	8·1
8	,, ,, ,, . . .	November .	..	8·4
9	,, Permanent manural plot .	April . .	7·4	..
10	,, ,, ,, ,, .	October .	11·8	8·15
11	Karnal	February .	11·4	7·8

TABLE I—*contd.*

Soil samples for isolation of fungi

No.	Source	Date	Moisture per cent	pH
	Neutral soils			
12	Karnal	October .	9·6	6·9
13	Simla	August .	23·5	6·9
	Acid soils			
14	Darjeeling (1″-4″). . . .	June . .	..	6·3
15	„ (6″-8″)	„ . .	..	..
16	Mungpoo	August .	40·0	6·1
17	Ramgarh	„ .	33·0	6·5
18	Chaubattia	„ .	6·0	6·5
19	Kulu	„ .	10·5	6·5
20	Manali	„ .	23·5	6·3

EXPERIMENTAL

The present account summarises a very hasty survey of a number of soil samples taken in 1935, mainly from Pusa and from certain hill districts in Northern India. The sources from which samples were taken are shown in Table I, and from the first six of these two samples were taken, one in early summer and one in the wet season.

Method of sampling and dilution.—Samples were removed by means of a copper cylinder measuring six inches in length and one inch in diameter, sharpened at one end and extended into a handle at the other. Several such cylinders were made and sterilised for use as required. When a soil sample was to be taken, the loose surface soil was pushed aside, and a plug taken of the top 4—6 in.; this was repeated from six places a foot apart and the plugs of soil placed in a sterile stoppered jar. (Samples of soils 13—20 were taken with an ordinary large spatula). At the earliest opportunity, the contents of the jar were rubbed through a sterile sieve of wire mesh, well mixed together, and after removal of a portion for moisture determination, returned to the stoppered jar.

The moisture content having been determined, the equivalent of twenty-five grams of dry soil was weighed out and transferred to a one-litre Roux flask containing 250 c.c. of sterile tap water. The flask was then fitted with a rubber stopper—dipped in alcohol and flamed—and the contents shaken steadily for ten minutes. Three successive ten-fold dilutions were made by transferring with sterilised 10-c.c. pipettes to three lots of 90 c.c. of sterile water contained in 250-c.c. flasks. The second and third of the small flasks thus represented dilutions of 1 : 1,000 and 1 : 10,000. Duplicate one-c.c. samples of each of these dilutions were placed in Petri dishes and the agar medium added after cooling in the usual manner. The plates were inverted when set, and incubated at 25°C. In the absence of an available cool incubator, some of the earlier samples rose to summer laboratory temperature, which occasionally reached 35°C.

Media.—The medium principally employed was that recommended by Bisby [1933], *i.e.*, Dox's agar acidified by the addition of 0·5 c.c. of 12·5 per cent lactic acid to each 100 c.c. medium. The acid was sterilised separately, and added at the time of plating. This medium suppresses bacterial growth very effectively, and is convenient to prepare and use. For certain fungi, such as the Mucors, however, a sucrose medium is not suitable ; duplicate platings were therefore made on an alternative medium which was also acidified. At first malt extract agar was tried for the purpose, and later Quaker oat agar was used. Attempts to find a substitute for acidity in suppressing bacterial growth—such as the employment of a medium of high sugar content—proved unsuccessful.

Counts.—Approximate counts were made on the eight plates from each soil sample, and as a rule these fell consistently between 20,000 and 40,000 per gram. The only variation was in samples 14 and 15, where the soil just below the surface gave 40,000—50,000, and the deeper soil only 10,000—20,000 per gram. This is in agreement with usual experience that fungi are most plentiful in the top few inches of soil.

Types of fungi isolated

The fungi recorded below represent those types which occurred most frequently on the plates. It must again be emphasised that they represent only a portion of the total fungi present. Soils 11 and 12, for example, were known to contain *Rhizoctonia,* but no colony of this appeared on the plates. Mucors were infrequent, although the technique of "direct isolation" from particles of soil would doubtless have yielded more. No Basidiomycetes were recorded.

Isolations have been allotted to the correct genus whenever possible. Pending further data as to which soil types are really widespread in India, and which are merely fortuitous, species determination has not been pressed beyond the limits to which the general mycologist can safely go, without reference to specialists. At a later date, when more material is available, it may be possible to enlist specialist aid in the identification of *Penicillium* and *Fusarium* spp. isolated from Indian soils.

From the present samples, the genera most frequently isolated were *Aspergillus* and *Penicillium,* the former being predominant in the Pusa soils. Several workers, *e.g.*, Waksman [1917], have pointed out that *Aspergillus* is more abundant in warmer climates and *Penicillium* in cooler ones. Of the thirty-two Indian soil fungi described by Chaudhuri and Sachar [1934] twelve belong to the genus *Aspergillus.* In the following list the classification of Thom and Church [1926] is adopted.

Asp. nidulans Eidam occurred most abundantly, being found in samples 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15. The isolations differed considerably, but all produced abundant perithecia, containing ascospores (rough in some isolations, smooth in others) with a double equatorial frill. On mounting the perithecia in lactophenol and pressing gently on the coverslip, a characteristic purple colour is given by the spores. This species is recorded in Indian soils by Chaudhuri and Sachar, and by Thakur and Norris.

Asp. niger v. Tiegh. comes next in order of abundance, in soils 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 14. It has been recorded by Shaw, Thakur and Norris, Mason, and Chaudhuri and Sachar.

Asp. terreus Thom, a sand-brown form, was isolated from samples 1, 2, 3, 7, 8, 10, 11. It is recorded by Mason, and by Chaudhuri and Sachar.

Asp. ustus Bain. Isolations of a dark brown mould from samples 3, 6, 7, 9, 10 and 15 differed among themselves, but agreed most closely with this species.

Asp. fumigatus Fres., one of the most abundant species in most soils, was unexpectedly absent from all the plates made from alkaline soils, although recorded from Pusa by Shaw [1915]. It occurred, however, in samples 16, 18 and 19. It is also recorded by Chaudhuri and Sachar, and by Thakur and Norris, although the latter state that it has no cellulose decomposing activity, a statement that throws some doubt on their identification.

Other species of *Aspergillus* were less abundant than those already named ; *Asp. flavipes* B. et S., from samples 1 and 2, is recorded by Chaudhuri and Sachar ; *Asp. flavus* Link. (samples 5, 11, 15) by Thakur and Norris ; *Asp. sydowi* B. et S. (sample 7 only) by Chaudhuri and Sachar ; *Asp. tamarii* Kita (sample 3) by Mason ; while *Asp. ochraceus* (sample 5), and *Asp. chevalieri* Mang. (samples 7 and 15) do not appear to have been previously recorded for India. Three isolations of *Aspergillus* spp. (from soils 11 and 15) have not been identified.

Penicillium.—This genus was present in most of the soils, but was far more abundant in the acid soils from hill districts. Probably the lower temperature was the chief factor concerned.

The systematy of *Penicillium* is still far from satisfactory, and exact determination of even the well-known species is a matter of great difficulty ; beyond these are several hundred species or strains all regrettably similar in their morphology. For the present, the soil isolations recorded here are merely allotted to their main groups.

Monoverticillata from samples 1, 7, 10, 11, 13, 15, 16 and 17.

Biverticillata symmetrica from samples 10, 13, 14, 15 and 19, all giving purple or orange pigment.

Unclassified from samples 2, 3, 8, 11, 15, 17 and 19.

The remaining isolations, of the less abundant genera, may now be taken in their systematic order.

Phycomycetes

The Mucoraceae were comparatively infrequent on the plates made, and are probably less abundant than in temperate climates. The following types were found :—

Rhizopus arrhizus Fischer, from samples 3, 6, 9, 13 and 17. This is closely related to *Rhiz. nigricans* Ehrenb., but is somewhat lighter in colour, has less well-developed rhizoids, and is capable of growth at a higher temperature. It is recorded by Chaudhuri and Sachar.

Mucor spp. from samples 10 and 14.

Cunninghamiella sp., probably *C. echinulata* (Thaxt.) Sacc. was obtained from sample 1. *C. elegans* Lend. is recorded by Shaw.

Syncephalastrum sp. Single colony isolated from sample 14. This may have been an atmospheric contamination. (*Syncephalis* sp. was isolated from manure and may also be regarded as a potential soil organism in India.

Ascomycetes

Perithecial strains of *Aspergillus* and *Penicillium* have already been dealt with. The only other *Ascomycetes* were two genera of the Sordariaceae and *Byssochlamys* sp.

Chaetomium ? indicum Corda from samples 5, 9 and 10.

Sordaria sp. from sample 2.

? *Byssochlamys* sp. On plates from samples 5 and 16 there occurred a whitish growth, later producing ochre coloured masses of conglomerate asci each containing a varying number of spores 2·5-3·5 × 2·5-3 μ No conidial stage was found.

Fungi Imperfecti

The majority of genera isolated from soil belong to this group. Bisby [1933] states that of his isolations from Manitoba soils, over ninety per cent were Fungi Imperfecti.

Phomaceae

Phoma sp. was obtained from sample 5.

Melanconiaceae

Melanconium sp. from samples 5 and 7. Black spores 8-12 μ × 3·0-3·5μ. Waksman [1916] reports a species of *Melanconium* from soil with spores 6·2-8·6 × 2·5-2·8μ ; as in the present case, this species was found to be an active cellulose decomposer.

Moniliaceae

Monilia spp. A number of isolations (samples 1, 2, 3, 9, 10, 11, 15 and 17) could only be grouped under this somewhat vague genus. The species from 1 and 11 agrees well with *Monilia koningi* Oud., which is recorded by Chaudhuri and Sachar.

Trichoderma lignorum (Tode) Harz. was abundant in samples 16 and 20. It was not isolated from any of the alkaline soils. It is, however, reported in alkaline Sahara soils by Killian and Feher [1935]. This organism has been shown by Weindling [1932] to be parasitic on *Rhizoctonia solani* and certain other soil pathogens.

Dematiaceae

?Botryotrichum sp. (samples 1, 2, 7 and 10). Brown hair-like sterile hyphae present ; conidia subhyaline, lens-shaped to spherical, 6-8×4-5-μ.

?Mesobotrys or *Chaetopsis* sp., from sample 11.

Cladosporium herbarum Link was found in samples 1, 3 and 11 and other unidentified species of *Cladosporium* (here used to include *Hormodendron*) from samples 1, 10 and 11.

?Epochnium sp. isolated from samples 10 and 11. Both hyaline and dematiaceous conidiophores. Some two-celled spores seen.

Alternaria. Samples 5, 7, 11 and 14 yielded species of this genus, all belonging to the "*Alternaria tenuis*" group.

Helminthosporium sativum P. K. and B. was found in sample 11, and *H. tetramera* in sample 2.

Acrothecium lunatum Wakker from samples 10, 11 and 19. Recorded from Indian soils by Chaudhuri and Sachar.

Torula sp. An isolation from sample 15 is provisionally placed here.

Trichosporium sp. (from sample 7 only). Spore measurements agree with *T. fuscum* (Link.)

Dematium sp. (samples 11 and 15).

Coniothecium spp. (samples 3, 7 and 10).

Coniosporium sp. from samples 2 and 9. Spores black, 10-12 μ.

Tuberculariaceae

Fusarium spp. were isolated from the following : 3, 11, 13, 15 showed macrospores ; in 2, 4, 11, 14 and 19 only microspores were seen and some of these species would perhaps be better classified as *Cephalosporium.* No one type appeared to be prominent, and the species were not identified.

Myrothecium sp. from sample 1. Black sporodochia ; conidia 7-8 μ×2·5μ greenish black. This is probably *M. roridum* Tode reported from American soils.

Stilbaceae

Stysanus sp. One species, agreeing with *S. stemonites* (Pers.) Corda was obtained from a sample of Pusa soil not shown in Table I.

Unidentified.—Owing to the absence of spore structures, or through loss of original cultures, twelve isolations (samples 1, 4, 11, 14, 15, 16 and 17) have not been even approximately identified. Most of these gave a sparse whitish growth on agar, and some were probably identical.

Power of decomposing cellulose

Action on cellulose was tested by ability to grow on filter paper strips half immersed in tubes containing the following medium. Since the only carbon source was the filter paper, vigorous growth indicated power to attack cellulose, although the converse was not necessarily true in all cases.

Medium.—Aq. dest. 1,000 c.c. ; $NaNO_3$ 0·5 grm. ; K_2HPO_4 1·0 grm. ; $MgSO_4 . 7H_2O$ 0·5 grm. ; KCl 0·5 grm. ; $FeSO_4 . 7H_2O$ 0·01 grm.

The most active cellulose decomposes were : *Aspergillus fumigatus*, *A. ustus*, *Asp. terreus*, *A. ochraceus*, *Penicillium* spp. (mostly monoverticillate), *Chaetomium*, *Trichoderma*, *Melanconium*, *Alternaria*, *Acrothecium*, *Helminthosporium*, *Stysanus*, *Dematium*, *Trichosporium* and *?Epochnium*.

The following species grew on the filter paper, but less actively : *A. nidulans*, *A. flavipes*, *A. niger*, and *A. flavus* ; *Penicillium* spp., *Sordaria*, *Phoma*, *Monilia* spp., *?Byssochlamys*, *Fusarium* spp., *Coniothecium* and *Botryotrichum*.

Summary

Two hundred isolations from Indian soils have been made and allotted to their approximate systematic position ; about thirty genera are represented.

The most striking feature is the predominance of *Aspergillus* spp. in most of the soils examined, and the limited distribution of such common soil types as *Aspergillus fumigatus* and *Trichoderma* spp.

The types most active in cellulose decomposition are recorded.

Acknowledgment

Considerable assistance was afforded by Mr. R. R. Sen in the examination of the cultures.

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STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

*IV. COLOUR CHANGES AND SEXUAL BEHAVIOUR IN DESEXUALISED *SCHISTOCERCA GREGARIA* ADULTS

BY

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(Received for publication on 18th January 1936)

INTRODUCTORY

Many species of locusts exhibit more or less marked colour changes during the adult stage. Lefroy [1906] described these changes in *Patanga succincta*, Uvarov [1911] in *Locusta migratoria*, La Baume [1918] in *Dociostaurus maroccanus*. Künckel had previously described this phenomenon in *Schistocerca gregaria*. Uvarov [1928] has summarised these colour changes in the above species, and with regards to *Schistocerca gregaria* he says :—In this species the adults for about a month after reaching that stage are of a wine-rose colour, which gradually changes to a brownish yellow ; the intensity of the latter increases and fully mature individuals are of a brilliant yellow, the males being particularly brightly coloured, while the females are somewhat darker and have a greyish tint." According to Uvarov [1928] : " The extensive physiological changes that occur during the development of the genital products find their external expression in changes of coloration ". The fact that, as a rule, the sexual maturity can be determined by the intensity of yellowing lends support to this view.

The best method of studying the influence of the sex-glands, on the change of coloration, is to remove them and note the effect. This is the line along which we worked.

The great practical importance of the phenomenon of change of colour is evident, because the colour of the fliers in a swarm provides an index to the degree of " ripness " of locusts for copulation and oviposition.

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*Studies on *Schistocerca gregaria* Forsk,

I. The Micropyle in *Schistocerca gregaria* Forsk., and some other Acridiidae. *Ind. J. Agric. Sci.* **3** : 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6** : 188-262.

III. Why Locusts Eat Wool ? A study in the hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6** : 263-67.

This investigation formed part of the Locust Research Scheme financed by the Imperial Council of Agricultural Research, India. The generosity of the Council is gratefully acknowledged.

TECHNIQUE

A small slit was made, with a pair of fine scissors, in the pleural membrane of the 6th abdominal segment and the gonads removed by means of forceps. The instruments were properly sterilised. The opening was sealed with ordinary paraffin wax. It was found convenient to make this cut in the left side of the insect.

TABLE I

Longevity of desexualised males and females

Cage No.	No. of adults	Date of acquiring wings (1930)	Date of operation (1930)	Date of death (1930-31)	Total life in days	Remarks
			(A) *Overwintered brood (1930-31)*			
5	1 Male.	4th Oct.	11th Oct.	20th Oct. 1930	16	No females were provided.
5	3 Males	Do.	Do.	27th Oct. 1930	23	
5	2 Males	Do.	Do.	3rd Nov. 1930	30	
5	1 Male	Do.	Do.	3rd Dec. 1930	60	
5	1 Male	Do.	Do.	31st Mar. 1931	178	
5	1 Male	Do.	Do.	8th April 1931	186	
6	1 Female	Do.	8th Oct.	9th Oct. 1930	5	No males were provided.
6	3 Females	Do.	Do.	10th Oct. 1930	6	
6	2 Females	Do.	Do.	27th Oct. 1930	23	
6	1 Female	Do.	Do.	17th Nov. 1930	44	
6	1 Female	Do.	Do.	19th Nov. 1930	46	
6	1 Female	Do.	Do.	29th Nov. 1930	56	
6	1 Female	Do.	Do.	26th Nov. 1930	83	
6	1 Female	Do.	Do.	29th Mar. 1931	176	
6	2 Females	Do.	Do.	5th April 1931	183	
6	1 Female	Do.	Do.	24th April 1931	202	
11	1 Female	16th Oct.	16th Oct.	9th April 1931	175	Castrated males were provided and copulation occurred.
19	1 Female	4th Oct.	17th Oct.	4th May 1931	212	
12	1 Female	16th Oct.	16th Oct.	9th April 1931	175	
34	1 Male	4th Oct.	11th Oct.	4th April 1931	182	Normal females were provided. Copulation and egg-laying took place.
35	1 Male	Do.	Do.	18th April 1931	196	
11	1 Male	Do.	19th Oct.	30th April 1931	208	Desexualised females were provided. Copulation occurred.
12	1 Male	16th Oct.	16th Oct.	20th May 1931	216	
14	1 Male	Do.	Do.	16th April 1931	182	

TABLE I —*contd.*

Cage No.	No. of adults.	Date of acquiring wings (1930)	Date of operation (1930)	Date of death (1930-31)	Total life in days	Remarks
			(B) *Spring brood (1931)*			
I 17	1 Female .	18th—25th May	31st May .	28th June . .	8±2	Normal male was provided on 2nd June 1931.
I 18	1 Female .	18th—25th May	Do. .	16th July . .	55±3	Normal male was provided on 2nd June 1931. Copulations were observed.
I 19	1 Female .	18th—25th May	Do. .	5th June . .	14±4	Normal male was provided.
I 19	1 Female .	2nd—8th June	10th June	5th Aug. . .	61±3	
I 23	1 Female .	18th—25th May	31st May .	18th June . .	28±3	Castrated male was provided.
I 20	1 Female .	20th May to 5th June	5th June .	22nd June . .	8±8	Normal males were provided.
I 20	1 Female .	21st June .	23rd June.	3rd July . .	12	
I 21	1 Female .	25th May to 5th June	5th June .	23rd July . .	56±8	
I 25	1 Female .	20th May to 5th June	8th June .	28th June . .	31±8	

TABLE II

Longevity of normal adults

Cage No.	Sex	Date of acquiring wings	Date of death	Total life (in days)
		(A) *Overwintered brood (1930-31)*		
		(1930)	(1931)	
39 a	Female	16th Aug. to 6th Sept.	13th April . .	230±10
47 a	Female . .	6th-7th Oct. . .	25th April . .	203±2
47 a	Male . .	2nd-7th Oct. . .	25th April . .	203±2
47 b	Female . .	Do. . .	13th April . .	191±2
47 b	Male . .	Do. . .	30th April . .	208±2
48 a	Female . .	Do. . .	19th April . .	197±2
48 a	Male . .	Do. . .	26th April . .	204±2
49 a	Female . .	27th-31st Oct. .	5th April . .	158±2
49 a	Male . .	Do. . .	26th Feb. . .	120±2
		(B) *Spring brood (1931)*		
Q 1	Female . .	11th-15th July .	11th Sept. . .	60±2
Q 1	Male . .	Do. . .	31st Aug. . .	49±2
ff 5	Female . .	26th-29th June .	16th Aug. . .	51±2
ff 5	Male . .	Do. . .	22nd Aug. . .	57±2
ff 7	Female . .	12th-16th July .	30th Aug. . .	49±2
ff 7	Male . .	Do. . .	6th Sept. . .	56±2
ff 11	Female . .	16th-26th July. .	30th Sept. . .	76±5
ff 11	Male . .	Do. . .	20th Sept. . .	66±5

All the operated males that had copulated were dissected at death and minutely examined. In no case was any trace of the testes found and we concluded from this that the removal was complete and the organ had not regenerated in any case.

Longevity of desexualised adults

Table I gives data regarding the longevity of the adults which had been operated upon. It will be noticed that in the case of the overwintered brood about sixty per cent of the individuals died within the first two months or so of the operation, but the remainder survived for fairly long periods extending to 216 days. These figures compare very favourably with those of the longevity of the normal overwintering locusts of which a few representative cases are given in Table II.

Similarly, of the desexualised locusts of the spring brood, which were bred in the laboratory in 1931, about fifty per cent died within the first fortnight of the operation, while the remainder survived from twenty-five to sixty-four days. This again compares favourably with the longevity of normal locusts of the spring brood (Table II). It is evident, therefore, that the total extirpation of the sex-glands does not essentially interfere with the general life of the individuals.

Colour changes

The overwintered insects, which had been operated upon, were greyish pink at the time of acquiring wings (October 1931), but as they advanced in age, they began to exhibit the characteristic colour changes. At first the bases of tegmina began turning yellow, and the deposition of the yellow pigments was then noticed on the abdominal segments. By about the middle of April most of the adults had developed bright yellow colour all over the body and this was particularly so in the male. This change in colour took place independent of the fact as to whether the adults were kept isolated (to simulate conditions of the solitary phase) or in twos or threes *i.e.* crowded (to simulate the conditions of the swarming phase).

As stated above it is generally believed that the development of the yellow pigment is connected with the maturation of the gonads. According to Vosseler [1905] ' the appearance of the yellow tint in the African Locust must be connected with the dissolution of the fat body, the yellow pigment being a bye-product that remains after the reserve material from the fat-body is used for building up the genital products [Uvarov, 1928].' Bücher, La Baume and others [1918], however, are of opinion that ' there is no direct physiological connection between the colour changes and the development of the genital products'. Their main argument is, that whereas the yellowing takes place simultaneously in both sexes, the female matures later than the male. They regard that the change in colour arises from senility rather than the development of the sex-glands. Our experiments have clearly shown that the development of yellow colour can take place even in the absence of the sex-glands.

Attention may also be drawn to another fact. Even the hoppers of *Schistocerca gregaria* in the gregarious phase may have intense yellow as the general colour of their body, black pigment in the cuticle forming a pattern over it. At the same time it has to be accepted that generally, if not always, the adults of the gregarious phase do develop yellow colour when sexually mature.

Sexual behaviour of desexualised adults

The adults of which the gonads had been removed acted as normal individuals in their sexual behaviour. Indeed, they displayed marked tendency for copulation, which continued throughout the breeding period *i.e.* from the middle of January to the end of April. In one instance a female, without ovaries, was first provided with a normal male with which she was observed to copulate ten times. Subsequently she was provided with a male which had been castrated and they were observed to copulate fifty-five times. This female lived for 175 days and her castrated mate for 208 days. Similarly in another case where the ovary-less female had survived for 212 days, she was observed to copulate with a castrated male sixteen times. The duration of coitus ordinarily lasted from thirty minutes to five hours.

It is thus evident that the removal of the sex-glands does not inhibit the copulation reflex, on the other hand, the act which remains unconsummated is repeated oftener and is prolonged in duration, showing that sexual apetite is greatly intensified.

It has been observed that generally the females which have not copulated do not oviposit, but occasionally such females do lay eggs, which develop parthenogenetically. Parthenogenesis is by no means rare among locusts. It is a significant fact that the normal females mating with castrated males oviposited as frequently as the normal females mating with normal males, but when compared with the normal females that had never mated, normal females matured when the desexualised females laid a larger number of egg packets. This shows that while the sexual act, perhaps, provides the necessary stimulus for the processes connected with oviposition, the actual transference of sperms from the male to the female is not necessary to set up the reflex.

Summary

Desexualised adults can live as long as the normal adults, and develop yellow pigment to the same degree as the normal insects. Their sexual behaviour remains unaltered. Thus for the development of the yellow coloration the presence of sex-glands is not necessary.

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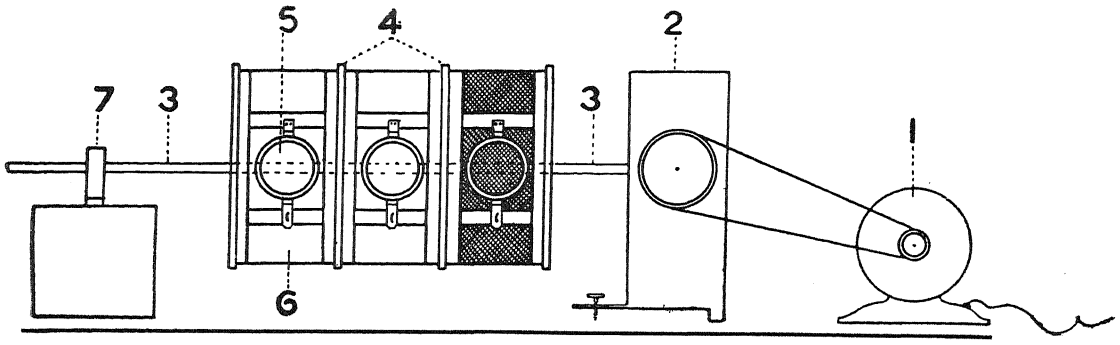

FIG. 1. REVOLVING WIRE GAUZE DRUMS :—1. electric motor ; 2. gear box ; 3. horizontal bar carrying the drums ; 4. glass sides of the drums ; 5. entrance to the drums ; 6. wire gauze side of the drum ; 7. ball-bearing support for the horizontal bar at its free end.

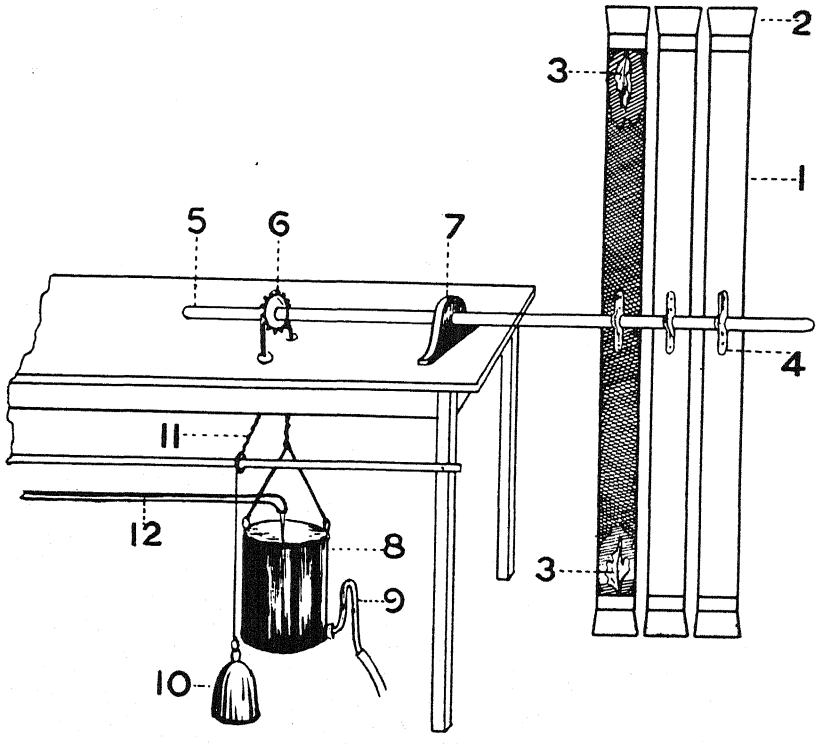

FIG. 2. SOMERSAULT CYLINDRICAL CAGES :—1. wire gauze cylindrical cage ; 2. cork stopper ; 3. leaf pinned for feeding ; 4. clip holding the cage to the horizontal bar, 5. ; 6. free-wheel ; 7. ball-bearing support ; 8. bucket with siphon arrangement, 9, at the bottom ; 10. the 'weight'; 11. bicycle chain attached to the weight and the bucket ; 12. water pipe running to the bucket.

STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

* V. PIGMENTATION AND PHYSICAL EXERTION

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(With Plates XXXIII-XXXV)

CONTENTS

	PAGE.
I. INTRODUCTION	591
II PREVIOUS WORK	593
III. PHYSICAL EXERCISE THROUGH MUSCULAR EXERTION	594
(A) Revolving wire-gauze drums.	554
(B) Somersault cylindrical cages.	595
(C) Observations	596
(D) Remarks	599
IV. PHYSICAL EXERCISE THROUGH ASSOCIATION WITH GRASS-HOPPERS OF OTHER SPECIES	608
V. DISCUSSION	622
VI. SUMMARY	623
REFERENCES	623

I. Introduction †

The most characteristic feature by which the hoppers of the gregarious phase and those of the solitary phase stand out from each other is their coloration.

† Studies on *Schistocerca gregaria* Forsk.

I. The Micropyle in *Schistocerca gregaria* Forsk, and some other Acridiidae. *Ind. J. Agric. Sci.* **3** : 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6** : 188-262.

III. Why do Locusts Eat Wool? A Study in the Hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6** : 263-67.

IV. Colour Changes and Sexual Behaviour in Desexualised *Schistocerca gregaria* Adults. *Ind. J. Agric. Sci.* **6** :586-90.

† This contribution is one of the series based on the work done under the Locust Research Scheme of the Imperial Council of Agricultural Research, India.

A '*gregarious*' or a swarming phase hopper, or a hopper bred crowded, is distinguished by the presence of a black pattern, which is absent in a '*solitary*' or a non-swarming phase hopper, or a hopper, bred in isolation the latter being a shade of green in colour. It is well known that one can change at will the coloration of a hopper born as a non-swarming phase hopper into the coloration typical of the swarming phase hopper, by breeding it under crowded conditions. And, conversely, one can change the coloration of a swarming phase hopper into the coloration of a typically non-swarming phase hopper by breeding it in isolation.

In nature the hoppers of the swarming phase live a very active life. They spend a major portion of the day, and under certain conditions even a portion of the night*, actively marching in band formations. Even during the periods of 'halt' they are in a state of agitation on account of excessive crowding. On the other hand, a non-swarming phase hopper spends its life in retirement in bushes and moves about only a little.

It appears that the presence of black pigmentation on the various parts of the body of a hopper and its active mode of living go hand in hand, while the absence of the black pattern is associated with an inactive mode of life. One depends upon the other and one leads to the other. For instance, the development and retention of the black pigment depends upon the activity of the individual, and it is the black pigmented hoppers which are more active. In the case of a non-swarming hopper, that causal factor—active life—being absent, the black pattern fails to appear. If that be the case, then a non-swarming hopper or a hopper bred isolated, if forced into activity, would, in spite of its solitary life, acquire the black pattern which is normally associated with a crowded life.

We arranged to force hoppers into this activity in two different ways. In one case the isolated hoppers of the non-swarming type were made to march a certain distance every day in specially devised apparatus. The second method simply consisted in keeping the isolated † non-swarming hoppers constantly in association with the hoppers or adults of some common grasshoppers and other Orthoptera.

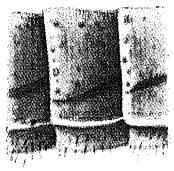
The coloration of the hoppers from stage to stage in the experiments given in this paper has not been described in detail. Instead, to express the extent and intensity of black pigmentation, we have used certain terms. Typical cases are illustrated by coloured drawings. The terms employed are: '*Green type*', '*Intermediate*' and '*Black type*'.

'*Green type*' indicates a hopper which is completely free from black pigmentation and has green body colour. The five stages of green type hoppers are illustrated by Figs. 1 to 5 (Plate XXXIV). On the other hand a hopper having black pigmentation typical of the hoppers in a swarm, or in a crowd

* Swarms of locust hoppers are known to continue their diurnal marches during hot and moonlit nights.

† Isolated from members of its own species.

TYPICAL GREEN HOPPERS



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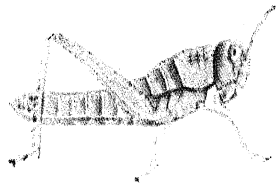
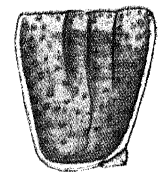


FIG. 1.



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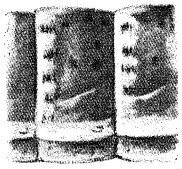

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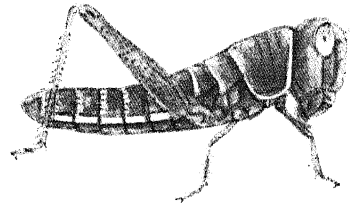
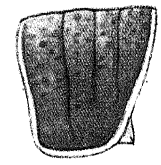


FIG. 2.



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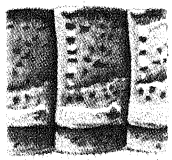

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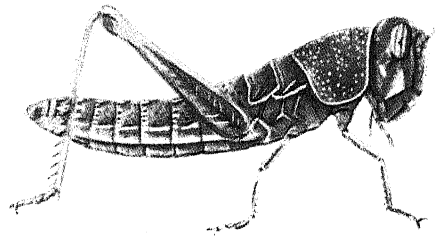
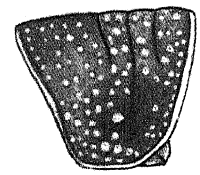


FIG. 3.



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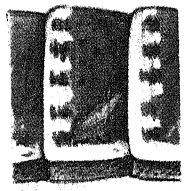

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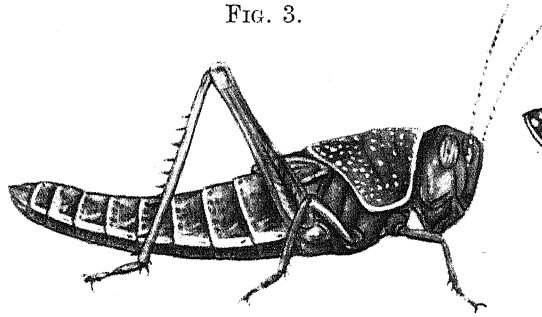

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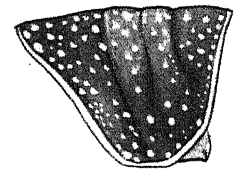

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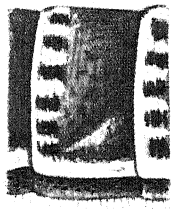

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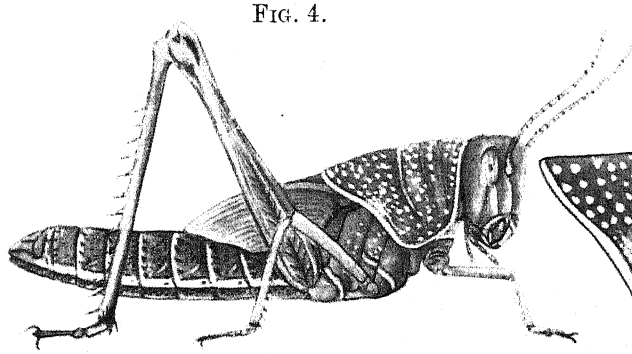
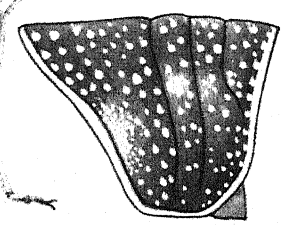


FIG. 5.



5*a*

FIG. 1. I stage (×2·5); FIG. 2. II stage (×2·5); FIG. 3. III stage (×1·1); FIG. 4. IV stage (×1·5); and FIG. 5. V stage (×1·1); with enlarged views of the pronotum and some abdominal segments

TYPICAL BLACK HOPPERS

1*a*

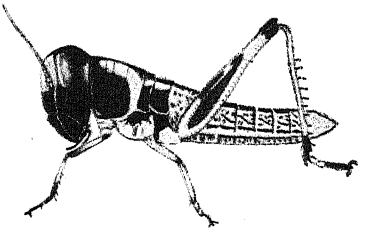


FIG. 1.

1*b*

2*a*

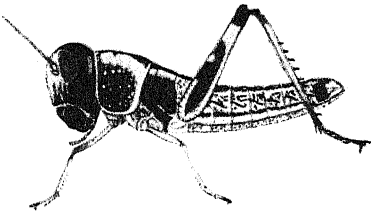
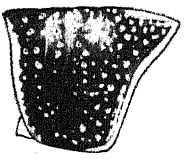


FIG. 2.

2*b*



3*a*

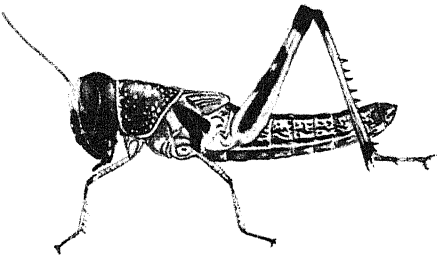
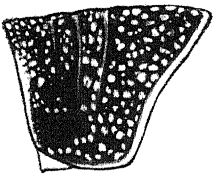


FIG. 3.

3*b*



4*a*

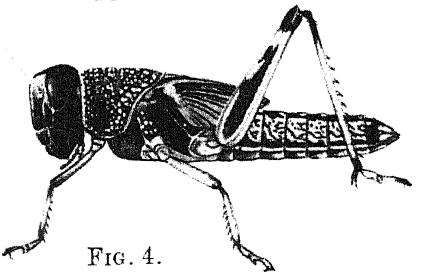
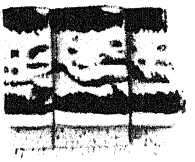
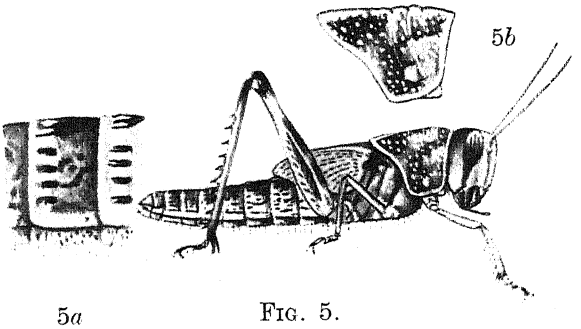


FIG. 4.



4*b*



5*b*

5*a*

FIG. 5.

FIGS. 1—4. Typical black hoppers: FIG. 1. II stage (×2·2); FIG. 2. III. stage (×1·3); FIG. 3. IV stage (×1·1); and FIG. 4. V stage (×0·8); with enlarged side views of the pronotum and some abdominal segments.

FIG. 5. An Intermediate hopper of the V stage (×0·8) with detailed side views of the pronotum and some abdominal segments.

(in the optimum temperature of about 30°C.) has been called '*Black type*'. *Black type* hoppers of 2nd to 5th stages are illustrated by figs. 1 to 4 (Plate XXXV). The term '*Intermediate*' indicates that state of black pigmentation which is midway between the '*Black type*' and the '*Green type*'. Thus a typical fifth stage '*Intermediate*' hopper appears like fig. 5 (Plate XXXV). Since all shades of black pigmentation between the '*Black type*' and the '*Green type*' appear, it has been found necessary to make further differentiations. Hoppers having more of black than the *Intermediate* have been described as '*Intermediate nearer black type*', while those having less of black have been described as "*Intermediate nearer green type*".

We have great pleasure in acknowledging the help of Mr. Munawwaruddin Siddiqi, Artist of the Entomological Section, Lyallpur, who prepared the plates for us.

II. Previous work

So far as we have been able to ascertain, only two investigators have attempted to test the influence of physical exertion on the development of black pigmentation in locust hoppers. Plotnikov [1927] worked on *Locusta migratoria* (Migratory Locust) and Faure [1932] on *Locustana pardalina* Walker (the Brown Locust).

Faure [1932] tried to force the hoppers of the Brown Locust into activity by a number of contrivances, of which his "Jarring apparatus" yielded some results. Of the 342 isolated hoppers that he subjected to "muscular exercise" only twenty-six (7·6 per cent) survived to the fifth stage. Of these only thirteen hoppers (15 per cent) maintained *solitaria* coloration, 11 hoppers (42 per cent) turned into *transience* and only 2 hoppers (7·7 per cent of the hoppers that reached V stage and 0·58 per cent of the total number of hoppers) acquired the '*gregaria*' coloration.

His experiments in which he crowded the hoppers of the Brown Locust with those of the other species are open to criticism. He placed a number of hoppers (six or seven) of *Locustana pardalina* in each cage in association with other hoppers. Six or seven hoppers of a species in a cage of the size he used (18 in. × 9 in. × 15 in.) would by themselves constitute a 'crowd' dense enough for the development of the characters of the phase, '*gregaria*'.

Although Plotnikov's experiments [1927] had yielded negative result, yet a brief review of his work will not be out of place.

To determine whether the excretory products were responsible for the development of the swarming coloration, Plotnikov, following Uvarov's advice, fed solitary hoppers of *Locusta migratoria* and *L. danica* on vegetation abundantly moistened with water extract of larval excreta. The result was negative.

To test if mechanical stimulation, caused by reciprocal jostling in a crowd, was instrumental in the production of the black pigment, he forced hoppers, which were reared isolated, to move for about two hours in glass globes fixed on a

revolving wheel. However, 'the difficulty in judging the exact moment of moulting, when the hopper should be in a state of rest, as well as technical difficulties ', did not permit him 'to attain that state of mechanical irritation in the experiment which is received by a hopper moving in a swarm '. The result of this experiment also was negative.

In the following year (1926) he caused mechanical irritation to be applied for several minutes daily to solitary hoppers, in those regions in which the black patterns are present in crowded hoppers, by means of soft silk cord-like brushes fixed on a rapidly revolving small wheel. The moulting time was avoided. He carried on the experiment up to the fourth stage when the experiment was discontinued, because he saw no hope of obtaining the result he expected. He concludes: " Thus my idea as to the swarming coloration being caused by mechanical irritation of the skin must be given up ".

He sought for an explanation for the production of black pigment in starvation, cannibalism, temperature and humidity, but from his experiments he concluded that none of these factors caused the deposition of black pigment.

III. Physical exercise through muscular exertion

The construction of our apparatus, designed to force activity into the life of the hoppers bred in strict isolation, was based on the following characteristic behaviour of the hoppers of the Desert Locust.

It is a matter of common observation that these hoppers when placed in a breeding cage mostly remain near the top of the cage and towards the source of light. They also show a tendency to keep their heads pointed away from the ground. Bodenheimer [1930] has also described this behaviour. Taking advantage of these tendencies we used revolving wire gauze drums and somersault cylindrical cages, described below, to force hoppers into muscular exercise.

(A) Revolving wire-gauze drums

The " revolving wire-gauze drums " (Plate XXXIII, fig. 1) consisted of six wire-gauze cylinders, four inches in diameter and three inches long. The two flat sides of the cylinders were of glass sheets *, each with a hole in the centre. Through these holes the cages were fixed on a horizontal bar, which was slowly revolved by means of an electric motor. The wire-gauze side was provided with a small circular entrance and door. The apparatus was so placed in the room and the rotation was so adjusted that the drum revolved from above downwards towards the brighter side of the room.

* At first cardboard sides were provided for the drums. These forming a convenient parking place for the hoppers when the apparatus was worked, told upon the efficiency of the apparatus and had to be replaced by glass sheets.

A hopper placed in a cage of this type behaved in the following manner :— When the apparatus was not working the hopper approached the brighter side and climbed up the side of the drum to keep its head upwards. As the apparatus was worked the drum moved from above downwards. The hopper was thus carried down and brought in a darker place and in a horizontal position. To bring itself to the original position, the hopper marched upwards. But this attempt was constantly nullified by the slow and continuous movement of the drums with the result that the hopper continued to move on and on. Unfortunately all the hoppers, particularly in earlier stages, did not respond with the same readiness. It was, therefore, found necessary to introduce small pieces of pith or cork in each drum to act as 'whips' for the sluggish ones. The speed of revolution of the drums was adjusted to avoid over-carriage of the hoppers.

The apparatus was worked only during the day, between ten and seventeen hours, with an interval between thirteen and fourteen hours for rest and feeding. A drum revolved about fifty times an hour and its circumference was about 12·5 inches. Each hopper therefore could have a maximum walk of about 312 feet a day. The average distance walked was, however, shorter than this.

During the period that the drums were not revolving, the hoppers were either supplied with cotton or cabbage leaves placed in the drums, or they were transferred to these plants in pots. They were, however, kept in strict isolation. (Details are given under each experiment.)

(B) Somersault cylindrical cages

The use of bits of pith or cork, in revolving drums, however, brought in another factor, namely, the stimulus of contact. In order to avoid this we devised the following apparatus which worked more satisfactorily and gave more convincing results than the "revolving drums".

In this case also advantage was taken of the positively phototropic and negatively geotropic responses of the Desert Locust hoppers. The apparatus (Plate XXXIII, Fig. 2) consisted of wire-gauze cylindrical cages (3 feet long and 2 inches in diameter) arranged vertically and fixed to a horizontal bar along their middle. The horizontal bar was attached to a bicycle free-wheel over which ran a bicycle chain. One end of the chain was fixed to a bucket. This bucket had a siphon arrangement and when the level of water in the bucket reached the siphon level, the siphon began to work and emptied the bucket very quickly. To the other end of the chain was fixed a 'weight' which was heavier than the empty bucket,

but lighter than the bucket with water at the siphon level. The bucket was placed under a tap and water dripped into it. When the water in the bucket reached the siphon level, the bucket became heavier than the 'weight' and went down, pulling the chain and thereby turning the horizontal bar round and rotating the cylindrical cages, through 180°. Soon after, the siphon started work, the bucket was emptied and the 'weight' went down pulling the bucket up. This movement, however, did not effect the horizontal rod and the cylindrical cages on account of the free-wheel arrangement. The cylindrical cages thus turned one way only, namely, with every downward movement of the bucket.

The open ends of these cylindrical cages were fitted with cork-stoppers, to each of which was pinned a green leaf, which served as food. The apparatus was set up in a dark room and near the top-ends of the cages was placed a 100 candle power electric lamp. The timing for the rotation of the cages was controlled by the speed with which the water dripped. After every twenty minutes or so the cages turned over.

The apparatus had several advantages over the "revolving drums". In the first place there was no foreign matter in the cages, and so the stimulus of contact was altogether eliminated. Secondly there was a provision for a constant supply of food inside each cage. The third very important advantage was that the interval between two turnings, even if it happened to be shorter than the period during which a hopper was able to traverse the whole length of the cylindrical cage, did not adversely affect the normal activities of the individual; because: (*i*) the hopper, irrespective of its position in the cage, took a turn for the upper and more lighted end of the cage whenever the cage took the somersault; and (*ii*) it was in a position to take rest whenever required and could moult without anything striking against its body. The last is particularly important, because an interference at the critical stage of moulting invariably results in death and it is this fact which accounts for the early deaths in a large number of cases in the 'revolving drums'. Lastly the apparatus could be worked continuously without constant attention and the hoppers did the maximum walking they were capable of doing.

(C) Observations

Experiment 1 (Revolving drums)

The hoppers in this experiment were taken from two hatchings.

Previous history.—The '*solitaria*' adults of both the pairs (P54 and P57) were collected at Pasni (Mekran coast) on 8th August 1932, and were received at Lyallpur on 19th August 1932. They were reared in pairs in wire-gauze cages (9 in. × 9 in. × 9 in.).

Female P54 laid eggs on 31st August 1932, which hatched on 14th September 1932. The hatching consisted of sixty-two green hoppers.

Female P57 laid eggs on 31st August 1932, which hatched on 14th September 1932. The hatching consisted of sixty-four green hoppers.

Thus all the hoppers had distinct '*solitaria*' tendency.

Conditions.—The experiment was started on 14th September 1932, and was conducted in the rearing room, the temperature during the experiment being :—

Highest maximum—35°C.	Highest minimum—32°C.
Lowest maximum—29°C.	Lowest minimum—24°C.
Average maximum—31·08°C.	Average minimum—27·13°C.

The food supplied was cotton leaves.

There was no pith or cork in the drums.

Experiment 2 (Revolving drums)

Previous history.—Reference No. of parents, P91.

The parents were collected at Sonmiani (Mekran Coast) and received at Lyallpur on 31st October 1932. They had marked "*solitaria*" characters and were reared in pairs.

The time of egg laying was not recorded. The hoppers hatched out on 1st February 1933—an all green hatching. Thus the hoppers had a distinct '*solitaria*' tendency.

Conditions.—The experiment was started on 1st February 1933 and was conducted in the hot house which was artificially heated. The temperature during the period was :—

Highest maximum—37·2°C.	Highest minimum—30·3°C.
Lowest maximum—27·2°C.	Lowest minimum—25·5°C.
Average maximum—32·05°C.	Average minimum—27·5°C.

The hoppers were removed from the drums after exercise and placed on cabbage plant for feeding. Pith and cork pieces were also placed in the drums.

Experiment 3 (Revolving drums)

Previous history.—Reference No. of parents, P136.

The parents were of the '*solitaria*' type and were received from Pasni on 8th August 1933. They were reared as a pair in the laboratory. The eggs were laid on 2nd August 1933 and the hoppers hatched out on 15th August 1933, the hatching consisting of forty-five green hoppers. Thus the hoppers had marked '*solitaria*' tendency.

Conditions.—The experiment was started on 15th August 1933 and was conducted in the rearing room, the temperature during the experiment being :—

Highest maximum—37·7°C.	Highest minimum—31·0°C.
Lowest maximum—28·0°C.	Lowest minimum—26·6°C.
Average maximum—30·87°C.	Average minimum—28·0°C.

Cotton leaves were supplied as food.

Pith and cork pieces were also placed in the drums.

Experiment 4 (Somersault cages)

Previous history.—Reference No. of parents, P110.

The parents were collected from Sonmiani where they were found in solitary state, but on receipt at Lyallpur on 31st October 1932 were described as "not having typical '*solitaria*' characters". They were reared in pairs.

The eggs were laid on 6th July 1932 and the hoppers hatched out on 30th December 1932. The hatching was a mixed one and consisted of fifty-eight green and seventeen intermediate hoppers. Pure green hoppers were selected for this experiment. Four of them (P110/2, P110/3, P110/4 and P110/5) were reared isolated on cotton plants during the first stage and were placed in the experimental cages after the first moult. They were green in the first as well as in the second stage. The fifth hopper (P110/6) was reared isolated on cotton plant during the first, second and third instars, and from the beginning of the fourth instar onwards it was reared in a cylindrical cage of the "somersault apparatus". During all the instars, including the fourth this hopper was of green colour.

Thus, in all cases, before subjecting the hoppers to experimental conditions, their *solitaria* tendency was established.

Conditions.—The experiment was started on 7th January 1933 and was conducted in a dark room artificially heated by means of electric heaters. The temperature during the period was :—

Highest maximum—35°C.	Highest minimum—28°C.
Lowest maximum—30°C.	Lowest minimum—24°C.
Average maximum—33°C.	Average minimum—26·5°C.

Cotton leaves were provided for feeding and were pinned on to the corks of the cylindrical cages. The leaves were changed thrice a day, at 9, 13 and 18 hours.

(D) Remarks

In Table I, has been described the extent of black pigmentation obtained by the hoppers from instar to instar in experiments (1) to (4). In experiment (1) the experimental hoppers did acquire black pigmentation, but not to the degree which was typical of the hoppers reared crowded at that temperature. Thus the blackest hopper of this experiment could only be described as '*Intermediate*'. In this experiment no pith or cork pieces were introduced into the cages and in the absence of this material, which acted as a sort of 'whip', the hoppers did not undergo the amount of physical exertion necessary for the development of the degree of black pigmentation typical of a '*Black type*' hopper at this temperature. We give the duration of the life cycle of these hoppers in Table II. The control hoppers remained green.

Pith and cork pieces were introduced in experiments (2) and (3) and black pigmentation obtained in these was more extensive and intensive than in experiment (1) (Table I). In fact, all the hoppers, in experiment (2) were described as '*Black Type*' in the last hopper stage, and all the hoppers in experiment (3) had acquired the degree of black pigmentation which was indeed very near the '*Black type*'. All the control hoppers were, of course, of the '*Green type*' throughout. Tables III and IV give the duration of life-cycle of the hoppers in experiments (2) and (3).

The fact that all the hoppers in experiment (4) (Somersault cages) acquired in the fifth stage the black pigmentation of the intensity of the '*Black type*' hopper is particularly significant, in view of the fact that one of the hoppers (P110/6) was forced into activity during one stage only, namely, the fourth stage, and the other four hoppers during three of the hopper stages. It may be pointed out once again that while these hoppers in experiment (4) were forced to march to their maximum capacity, the somersault apparatus working continuously, the hoppers in experiments (1), (2) and (3) were forced into activity for about six hours a day only. This explains the quicker appearance of the black pigmentation in experiment (4). The duration of the hopper stages of this experiment is given in Table V.

TABLE I

Extent of pigmentation from instar to instar of the isolated hoppers forced into activity

Experiment No.	Condition	Reference No. of hoppe s	I stage	II stage	III stage	IV stage	V stage	Remarks
1	Revolving drums (without pith or cork).	P54-57/E 1 .	Green type	Interm e d i a t e nearer green type.	Interme di a t e	Interme d i a t e (died).	...	
		P54-57/E 2 .	Do. .	Intermediate .	Interme d i a t e nearer green type (died).	...	...	
		P54-57/E 3 .	Do. .	Do. .	Do. .	...	...	
		P54-57/E 4 .	Do. .	Interme d i a t e nearer green type.	Do. .	Interme d i a t e nearer green type (died).	...	
	Control . .	P54-57/Cont. 1.	Do. .	Green type .	Green type .	Green type .	Green type .	
		P54-57/Cont. 2.	Do. .	Do. .	Interme d i a t e nearer green type.	Do. .	Do.	
		P54-57/Cont. 4.	Do. .	Do. .	Green type .	Do. .	Do.	
2	Revolving drums (with pith and cork).	P91/R 1 .	Do. .	Almost black type.	Black type .	Black type .	...	
		P91/R 2 .	Do. .	Intermedeiate .	Black type (Escaped).	...	...	
		P91/R 3 .	Do. .	Interme d i a t e nearer green type.	Black type .	Black type * (died).	...	* Hopper died during moult. The parts that were exposed showed that the hopper was black type.
		P91/R 4 .	Do. .	Do. .	(*a*) Black type (*b*) Black type (died).	...	...	(*b*) Extra moult.
	Control . .	P91/R 5 .	Do. .	Almost green	Green type .	Green type .	...	
		P91/R 6 .	Do. .	Do. .	Do. .	Do. .	...	

Experiment No.	Condition	Reference No. of hoppers	I Stage	II Stage	III Stage	IV Stage	V Stage	Remarks
3	Revolving drums (with pith and cork).	P136/15.8.33 A	Do. .	Intermediate nearer green type.	Intermediate nearer green type.	Intermediate nearer black type.	Intermediate nearer black type.	
		P136/15.8.33 B	Do. .	Intermediate nearer black type.	Intermediate .	Do. .	...	Escaped during the IV stage.
		P136/15.8.33 C	Do. .	Intermediate nearer green type.	Intermediate nearer green type.	Intermediate .	Intermediate nearer black type.	
		P136/15.8.33 E	Do. .	Do. .	Do. .	Do. .	Do.	
	Control . .	P136/15.8.33 1.	Do. .	...	...	Green type .	Green type.	
4	Somersault cages	P110/2 . .	Green type.	Green type .	*	Intermediate .	Intermediate nearer black type.	During I instar the hoppers were reared isolated on plants. After the 1st moult they were transferred into experimental cages.
		P110/3 . .	Do. .	Do. .	*	Black type .	Black type.	
		P110/4 . .	Do. .	Do. .	*	Do. .	Do.	* The colouration in the third instar was not described.
		P110/5 . .	Do. .	Do. .	*	Intermediate nearer black type.	Do.	
		P110/6 .	Do. .	Do. .	Green type .	Green type .	Almost black type.	This hopper was kept isolated on the plant during I, II and III instars. At the beginning of the fourth instar when it was transferred to experimental cage it was a pure green hopper.

TABLE II

Life history of hoppers in experiment 1

Reference :—No. P54-57.

Temperature :—Average maximum—31·08°C.
Average minimum—27·13°C.

Date	Experimental				Control		
	E 1	E 2	E 3	E 4	Cont. 1	Cont. 2	Cont. 4
14th Sept. 1932 .	I	I	I	I	I	I	I
15th ,, ,, .							
16th ,, ,, .							
17th ,, ,, .							
18th ,, ,, .							
19th ,, ,, .	II	II			II	II	II
20th ,, ,, .				II			
21st ,, ,, .							
22nd ,, ,, .			II				
23rd ,, ,, .							
24th ,, ,, .							
25th ,, ,, .					III		
26th ,, ,, .						III	III
27th ,, ,, .							
28th ,, ,, .	III						
29th ,, ,, .							
30th ,, ,, .			III	III			
1st Oct. ,, .		III					
2nd ,, ,, .		(D)					
3rd ,, ,, .							
4th ,, ,, .							

NOTE.—D indicates death of the hopper.

TABLE—II—*contd*

Dates	Experimental				Control		
	E 1	E 2	E 3	E 4	Cont. 1	Cont. 2	Cont. 4
5th Oct. 1932 .			(D)				
6th ,, ,, .							
7th ,, ,, .					IV		
8th ,, ,, .							IV
9th ,, ,, .						IV	
10th ,, ,, .							
11th ,, ,, .							
12th ,, ,, .	IV						
13th ,, ,, .				IV (D)			
14th ,, ,, .							
15th ,, ,, .	(D)				V	V	
16th ,, ,, .							V

NOTE.—D indicates death of hopper.

TABLE III

Life history of hoppers in experiment 2

Reference :—No. P91

Temperature of hot house :—Average Maximum—32·05°C.
Average Minimum—27·5°C.

Dates	Experimental				Control	
	R1	R2	R3	R4	R5	R6
1st Feb. 1933 . . .	I	I	I	I	I	I
2nd ,, ,, . . .						
3rd ,, ,, . . .						
4th ,, ,, . . .						
5th ,, ,, . . .						

TABLE—III—*contd*

Dates	Experimental				Control	
	R 1	R 2	R 3	R 4	R 5	R 6
6th Feb. 1933 . .	II				II	II
7th ,, ,, . . .			II			
8th ,, ,, . . .		II		II		
9th ,, ,, . . .						
10th,, ,, . . .						
11th,, ,, . . .						
12th,, ,, . . .						
13th,, ,, . . .						
14th,, ,, . . .					III	III
15th,, ,, . . .						
16th,, ,, . . .	III		III	III (*a*)		
17th,, ,, . . .		III				
18th,, ,, . . .						
19th,, ,, . . .		Escaped.				
20th,, ,, . . .				III (*b*)		IV
21st ,, ,, . . .						
22nd,, ,, . . .					IV	
23rd,, ,, . . .						
24th,, , . . .	IV					
25th,, ,, . . .				(D)		
26th,, ,, . . .						
27th,, ,, . . .	(D)		IV			
28th,, ,, . . .			(D)			

NOTE.—D indicates death of the hopper.
III (*a*) and III (*b*) represent the normal and the extra third stage.

TABLE IV

Life history of hoppers in experiment 3

Reference:—No. P136

Temperature of rearing room:— Average maximum—30·87°C.
Average minimum—28·0°C.

Dates	Experimental				Control
	A	C	E	B	I
15th Aug. 1933 . . .	I	I	I	I	I
16th „ „ . . .					
17th „ „ . . .					
18th „ „ . . .					
19th „ „ . . .					
20 h „ „ . . .					
21st „ „ . . .					
22nd „ „ .					
23rd „ „ . . .					
24th „ „ . . .	II		II		
25th „ „ . . .					
26th „ „ . . .				II	
27th „ „ . . .					
28th „ „ . . .					
29th „ „ . . .					
30th „ „ . . .					
31st „ „ . . .		II			
1st Sept. 1933 . . .					
2nd „ „ . . .					

TABLE IV—*contd.*

Dates	Experimental				Control
	A	C	E	B	I
3rd Sept. 1933 . . .					
4th ,, ,, . . .					
5th ,, ,, . . .					
6th ,, ,, . . .					
7th ,, ,, . . .					
8th ,, ,, . . .					
9th ,, ,, . . .					
10th ,, ,, . .					
11th ,, ,, . .					
12th ,, , . . .					IV
13th ,, ,, . . .					
14th ,, , . . .			IV	IV	
15th ,, ,, . . .					
16th ,, ,, . . .	IV	IV			
17th ,, ,, . . .					IV
18th ,, ,, . . .					
19th ,, ,, . . .					
20th ,, ,, . . .					A
21st ,, ,, . . .					
22nd ,, ,, . . .					
23rd ,, ,, . . .					
24th ,, ,, . . .			V		
25th ,, ,, . . .	V	V			
26th ,, ,, . . .					

TABLE V

Life history of hoppers in experiment 4

Reference :— No. P110.

Temperature of dark room :—Average maximum—33°C.
Average minimum—26·5°C.

Dates	Experimental				
	2	3	4	5	6
30th Dec. 1932 . .	I	I	I	I	I
31st „ „ . . .					
1st Jan. 1933 . . .					
2nd „ „ . . .					
3rd „ „ . . .					
4th „ „ . . .					
5th „ „ . . .					
6th „ „ . . .					
7th „ „ . . .	II	II	II	II	
8th „ „ . . .					
9th „ „ . . .					
10th „ „ . . .					
11th „ „ . . .					
12th „ „ . . .					
13th „ „ . . .					
14th „ „ . . .					
15th „ „ . . .					
16th „ „ . . .	IV	IV	IV	IV	
17th „ „ . . .					IV
18th „ „ . . .					

TABLE V—*contd.*

Dates	Experimental				
	2	3	4	5	6
19th Jan. 1933 . .					
20th „ „ . . .					
21st „ „ . . .					
22nd „ „ . . .					
23rd „ „ . . .	V	V	V	V	
24th „ „ . . .					
25th „ „ . . .					
26th „ „ . . .					V

IV. Physical exercise through association with grasshoppers of other species

Faure confined *Locustana pardalina* hoppers with those of *Locusta migratoria migratorioides*, and *Locusta migratoria* hoppers with the hoppers of *Schistocerca gregaria* or *L. pardalina*. In all the five experiments that he performed, he kept more than one hopper of the species under experiment in each cage. This makes the results inconclusive. The size of the cage, the number of experimental hoppers reared in each cage and the number of associate hoppers in Faure's experiments were as follows :—

Serial No.	Experimental hoppers		Size of cage	Associates		Results
	Name	No.		Name	No.	
1	*L. pardalina* .	7	18in. × 9in. × 15in. .	*L. m. migratorioides.*	400	Four reached V stage; all good gregaria type.
2	Ditto .	6	Ditto .	Ditto .	400	One survived to IV stage and was described as good gregaria.
3	*L. m. migratorioides.*	5	Ditto .	*Schistocerca gregaria.*	250	Two reached V stage; good transiens near gregaria type.
4	Ditto .	6	6in. × 4·5in. × 6in. .	*L. pardalina* .	65	One survived to IV stage, Gregaria type.
5	Ditto .	3	Ditto .	Ditto .	50	Ditto.

When more than one hopper of a species are confined in a small cage, as Faure did, it cannot be claimed that the *'gregaria'* characters obtained were not the result of intra-specific crowding.

In all the five experiments performed by us in each cage only one '*solitaria*' hopper of the Desert Locust was in association with a number of other Orthoptera. In three of these experiments the associate insects were the hoppers of *Poecilocerus pictus* ; in the fourth experiment a miscellaneous collection of grasshoppers (predominatingly *Chrotogonus* sp.) was associated. In the fifth experiment the associates were the adults of the house cricket (*Gryllus domesticus*). The last experiment is particularly interesting inasmuch as the association did not result in the production of the black pigmentation in the *solitaria* hoppers of the Desert Locust.

Experiment 5

Previous history.—Reference No. of the parents, K22.

The parents were found in solitary state at Angaria Gadap (near Karachi). They were collected on 30th February 1932 and were received at Lyallpur on 4th March 1932. They were bred as a pair, in a cage 9 in. ×9 in. ×9 in.

The eggs were laid on 26th April 1932 and hatched on 10th May 1932. The hatching was a mixed one. Only the '*solitaria*' hoppers were employed in this experiment. Thus the hoppers used were '*solitaria*' even though they belonged to a mixed hatching. The hoppers may be described as having a moderate '*solitaria*' tendency.

Associates.—The associates were the hoppers of *P. pictus*. At the start of the experiment three second instar hoppers of *P. pictus* were introduced in each cage. This strength was maintained throughout the experiment, the dead and the disabled ones being replaced from time to time with fresh hoppers from the field.

Conditions.—The cage was a wire—gauze cylinder six inches in height and five inches in diameter. It was fixed on a cabbage plant, the latter serving as food material. The experiment was conducted at room temperature which during the period was :—

Highest maximum—38·5°C.	Highest minimum—32°C.
Lowest maximum—33°C.	Lowest minimum—26°C.
Average maximum—35·7°C.	Average minimum—29·7°C.

Remarks.—From the record of the control isolated hoppers (K22/10.V.32, 1, 2, 3, 4, 5 and 7) it is clear that the *solitaria* hoppers of this batch, when bred isolated, maintained their '*solitaria*' coloration. This established the '*solitaria*' tendency of the hoppers of this experiment (Table VI). The hoppers that were associated with three *P. pictus* hoppers assumed gregaria coloration without exception. We can conclude, that in association with three *P. pictus* hoppers, in a space of the size of the cage employed in this experiment, a single hopper of *Schistocerca gregaria* assumes the coloration typical of a crowded hopper at this temperature. The progress of black pigmentation in these hoppers from instar to instar is given in Table VI. Table VII gives the life history of these hoppers.

TABLE VI

Progress of pigment formation in experiment 5

Reference No.	I stage	II stage	III stage	IV stage	V stage	Adult	Remarks
			Experimental				
K22/10-5-32 . 11	Pure green	Intermediate .	Almost black type.	Almost black .	Black type .	Gregarious .	One *Schistocerca gregaria* hopper with three *P. pictus*-hoppers.
K22/10-5-32 . 12	Do. .	Black type .	Black type .	Black type .	Black type .	Do. . .	One *Schistocerca gregaria* hopper with three *P. pictus* hoppers.
K22/10-5-32 . 13	Do. .	Black type .	Black type .	Almost black type.	Black type .	Do. . .	Ditto.
			Control isolated				
K22/10-5-32 . 1	Do. .	Intermediate nearer green type.	Intermediate nearer green type.	Intermediate .	Intermediate .	Solitary . .	Control Isolated.
K22/10-5-32 . 2	Do. .	Almost green type.	Green type .	Green type .	Green type .	Do. . .	Do. Do.
K22/10-5-32 . 3	Do. .	Almost green type.	Almost green type.	Green type	...	...	Do. Do.
K22/10-5-32 . 4	Do. .	Intermediate .	Almost green type.	Almost green type.	...	...	Do. Do.
K22/10-5-32 . 5	Do. .	Green type .	Green type .	Green type .	Green type .	...	Do. Do.
K22/10-5-32 . 7	Do. .	Almost green type.	Green type .	Green type .	Green type .	Solitary . .	Do. Do.
			Control crowded				
K22/10-5-32 . 8	Do. .	a. Black type . b. *	a. Black type	a. Intermediate nearer black type. ...	a. Intermediate nearer black type. ...	a. Intermediate ...	Do. Two in a cage *Died during the 1st moult.
K22/10-5-32 . 9	Do. .	a. Black type . b. Black type .	a. Black type . b. Black type.	a. Black type . b. Black type.	a. Black type . b. Black type.	a. Gregarious . b. Gregarious.	Control Two in a cage.
K22/10-5-32 . 10	Do. .	a. Black type . b. ... c. ...	a. Almost black	Intermediate .	Intermediate .	Intermediate .	Control. Three in a cage-1 died on second day, 2nd died at 1st moult, 3rd carried on to VI stage.

TABLE VII

Life history of hoppers in experiment 5

Reference No. K 22/10. V. '32

Temperature:—Highest maximum—38·5°C. Highest minimum—32·6°C.
Lowest maximum—33·0°C. Lowest minimum—26·0°C.
Average maximum—35·7°C. Average minimum—29·7°C.

Date (1932)	Experimental			Control, isolated						Control, crowded		
	11	12	13	1	2	3	4	5	7	8	9	10
10th May .	I	I	I	I	I	I	I	I	I	I (2)	I (2)	I (3)
11th ,, .												
12th ,, .												
13th ,, .												
14th ,, .												
15th ,, .				II								
16th ,, .	II	II	II					II			II (2)	I (2) II (1)
17th ,, .					II	II	II		II	II I (D)		1 (2) (D) II 1
18th ,, .												
19th ,, .												
20th ,, .	III	III	III	III				III			III	
21st ,, .					III	III	III		III			III
22nd ,, .										III		
23rd ,, .								IV			IV (2)	
24th ,, .	IV			IV		IV				IV		
25th ,, .		IV	IV		IV		IV		IV			IV
26th ,, .												
27th ,, .							Died.	V			V (2)	
28th ,, .				V	V					V		
29th ,, .	V					IV (Died)			V			V
30th ,, .		V	V									
31st ,, .												
1st June .												
2nd ,, .												
3rd ,, .										A 1		
4th ,, .			A 1	A 1	A 1						A 2	A 1
5th ,, .		A 1										
6th ,, .	A											
7th ,, .									A 1			

Note.—Numbers in brackets indicate the thickness of crowd.
D indicates death of the hopper.

Experiment 6

Previous history.—Reference No. of parents, K14.

The parents belonged to the same field collection as those in experiment (5).

The date on which the eggs were laid was not recorded.

The hoppers hatched out on 13th May 1932. The hatching consisted of fifty-four green hoppers only. Thus the '*solitaria*' tendency in the hoppers employed in this experiment was more marked than in the hoppers employed in experiment (5).

Associates.—*P. pictus* hoppers were the associates. Their strength was maintained at three throughout the experiment.

Conditions.—The hoppers were reared in cylindrical wire-gauze cages six inches long and five inches in diameter. In the laboratory the temperature during the period was :—

Highest maximum—38·8°C.	Highest minimum—32°C.
Lowest maximum—32·4°C.	Lowest minimum—28°C.
Average maximum—34·03°C.	Average minimum—29·01°C.

Remarks.—The experiment is similar to the previous experiment with regard to :—(*a*) space, (*b*) number and species of the associates, and (*c*) temperature.

Table VIII gives the stage-to-stage coloration of the hoppers. It shows that while the isolated hoppers in control continued to be '*Green type*' throughout the hopper stages, those in association varied from '*Green type*' to '*Black type*'.

It would seem that for a hopper, that belongs to a pure green hatching and is born of *solitaria* parents, an association with three *P. pictus* hoppers, in the space provided in this experiment, creates that 'neutral' state which may either lead to the production of black pigmentation or may remain ineffective through such minor individual differences as, (*i*) the slightly bigger or smaller size of the food plant affecting the available space for the movements of the hoppers, (*ii*) the small individual variations in the activity of the associates, and (*iii*) the individual difference in the physiology of the hoppers themselves. In experiments on crowding one finds that the extent and intensity of pigmentation depends upon the intensity of population per unit area of space. The life cycle of hoppers is given in Table IX.

TABLE VIII

Extent of black pigmentation from instar to instar of the isolated hoppers in experiment 6

Reference No.	I stage	II stage	III stage	IV stage	V stage	Adult	Remarks
K14/11	Green type	Black type	Black type	Black type	Black type	Gregaria	One *Schistocerca gregaria* hopper associated with 3 *P. pictus* hoppers.
K14/12	Do.	Intermediate nearer black type.	Intermediate	Intermediate nearer green type.	Almost green type.	Transient	
K14/13	Do.	Do.	Do.	Green type	Green type	Solitaria	
K14/14	Do.	Intermediate	Do.	Intermediate	Do.	...	
K14/1	Do.	Green type	Green type	Green type	Green type	Solitaria	Control. Isolated hoppers.
K14/2	Do.	Almost green type.	Do.	Do.	Do.	Do.	
K14/3	Do.	Do.	Do.	Do.	...	...	
K14/5	Do.	Green type	Do.	Do.	Green type	Solitaria	
K14/7	Do.	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Gregaria (b) Gregaria	Control. Two hoppers of *Schistocerca gregaria* in a cage.
K14/8	Do.	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Gregaria (b) Gregaria	Ditto.
K14/9	Do.	(a) Black type (b) Black type (c) Black type	(a) Black type (b) Black type (c) Black type	(a) Black type (b) Black type (c) Black type	(a) Black type (b) Black type (c) Black type	(a) Gregaria (b) Gregaria (c) Gregaria	Control. Three hoppers of *Schistocerca gregaria* in a cage.

TABLE IX

Life-history of hoppers in experiment 6

Reference :—No. K14.

Temperature :—Highest maximum—38·8°C. Highest minimum—32°C.
Lowest maximum—32·4°C. Lowest minimum—28°C.
Average maximum—34·03°C. Average minimum—29·01°C.

Date (1932)	Experimental				Control, isolated				Control, crowded		
	11	12	13	14	1	2	3	5	7	8	9
13th May .	I	I	I	I	I	I	I	I	1 (2)	1 (2)	1 (3)
14th ,, .											
15th ,, .					II						
16th ,, .											
17th ,, .											
18th ,, .	II	II	II				II		I (1) II (1)	II (2)	II (3)
19th ,, .				II		II		II	II (2)		
20th ,, .											
21st ,, .											
22nd ,, .							III				
23rd ,, .	III	III	III	III	III	III		III	III (2)	III (2)	III (3)
24th ,, .											
25th ,, .	IV	IV	IV	IV			IV		IV (1) III (1)	IV (2)	III (1) IV (2)
26th ,, .					IV	IV		IV	IV (2)		IV (3)
27th ,, .							Died				
28th ,, .											
29th ,, .											
30th ,, .	V	V	V	V	V					V (2)	IV (2) V (1)
31st ,, .						V		V			V (3)
1st June .									V (2)		
2nd ,, .											
3rd ,, .											
4th ,, .											
5th ,, .			A								
6th ,, .		A		V (D)		A			A (1) V (1)	A (1) A (1)	A (2) V (1)
7th ,, .	A				A			A		A (2)	
8th ,, .									A (2)		A (3)

Note.—Numbers in brackets indicate the thickness of crowd.

Experiment 7

Previous history.—The hoppers for this experiment were taken from two hatchings.

Reference Nos. of the parents, K5 and K17.

The parents were of the phase '*solitaria*' and belonged to the same collection as those in experiments (1) and (2). They were bred in pairs. The eggs were laid on 30th April 1932 and hatched on 19th May 1932. The hatchings from K5 and K17 consisted of fifty-seven and fifty-one green hoppers respectively. All the hoppers therefore had a marked '*solitaria*' tendency.

Associates.—During its first stage each *Schistocerca gregaria* hopper had six hoppers of *P. pictus* as its associates. Subsequently, however, the number of *P. pictus* hoppers varied from stage to stage and from hopper to hopper. The number of associates in each case is indicated in Table X.

Conditions.—Each *Schistocerca gregaria* hopper in company with its associate hoppers was reared on a cabbage plant in a cylindrical wire-gauze cage six inches high and five inches in diameter. The experiment was conducted in the laboratory and the temperature during the period was:—

Highest maximum—40°C.	Highest minimum—33°C.
Lowest maximum—34°C.	Lowest minimum—28°C.
Average maximum—36·5°C.	Average minimum—30·05°C.

Remarks.—The experiment was similar to experiment (6) with regard to (*a*) space and (*b*) '*solitaria*' tendency of the hoppers. The temperature in this experiment was slightly higher than in the previous experiments and was thus a little less favourable for the acquisition of '*gregaria*' coloration. The number of associates was generally higher. All the hoppers in an association of such thickness have assumed the '*gregaria*' coloration. The state of black pigmentation from instar to instar is indicated in Table X and the life cycle of these hoppers is given in Table XI.

TABLE X

Progress of black pigmentation in experiment 7

Ref. No.	I stage	II stage	III stage.	IV stage	V stage	Adult	Remarks
(Single *Schistocerca gregaria* hopper crowded with *P. pictus* hoppers)							
K5-17/1	*Green type P.* p. 6	*Black type P.* p. 6	*Black type P.* p. 6	*Black type P.* p. 8	*Black type P.* p. 4	Gregarious .	Male
K5-17/2 . . .	*Green type P.* p. 6	*Black type P.* p. 8	*Black type P.* p. 8	*Black type P.* p. 8	*Black type P.* p. 1	Almost gregaria .	Female
K5-17/3	*Green type P.* p. 6	*Black type P.* p. 6	*Black type P.* p. 8	*Black type P.* p. 8	Intermediate very near *black type P.* p. 4	Gregaria . .	Female
K5-17/5 . . .	*Green type P.* p. 6	*Black type ?* .	*Black type P.* p. 8	*Black type P.* p. 8	*Black type P.* p. 4	Almost gregaria .	Male
[Isolated *Schistocerca gregaria* hopper (control)]							
K5-17/6 . . .	Green type .	Green type .	...	...	...	...	
K5-17/7 . . .	Green type .	Green type .	Green type .	Green type .	Green type .	Solitaria . .	Female
[Crowded *Schistocerca gregaria* hoppers (control)]							
K5-17/8	*Green type S.* g. 4	*Black type S.* g. 4	*Black type S.* g. 4	*Black type S.* g. 3	*Black type S.* g. 3	Gregaria . .	

NOTE.—*P. p.*=*Peocilocerous pictus* hoppers.

S. g.—*Schistocerca gregaria* hoppers.

The number given below the colour of a stage show the strength of the crowd of the *P. pictus* hoppers or *Schistocerca gregaria* hopper, as specified, during stage. The effect of crowding appears in the next stage.

TABLE XI

Life—history of hoppers in experiment 7

Temperature:—Heighest maximum—40°C. Highest minimum—33°C.

Lowest maximum—34°C. Lowest minimum—28°C.

Average maximum—36·05°C. Average minimum—30·05°C.

Date (1932)	Experimental				Control		
	K5-17 / 1	K5-17 / 2	K5-17 / 3	K5-17 / 5	Cont. / K5-17/6	Cont. / K5-17/7	Cont. / K5-17/8
20th May .	I	I	I	I	I	I	I
21st ,, .							
22nd ,, .							
23rd ,, .		II					
24th ,, .	II		II	II		II	II(4)
25th ,, .					II		
26th ,, .							
27th ,, .	III	III	III	III			III(3) II(1)
28th ,, .						III	III(4)
29th ,, .							
30th ,, .				IV			III(3) III(D)
31st ,, .	IV	IV	IV		Died II		IV(3)
1st June .						IV	
2nd ,, .							
3rd ,, .							
4th ,, .			V	V			IV(2) V(1)
5th ,, .	V	V				V	
6th ,, .							
7th ,, .							
8th ,, .							
9th ,, .							
10th ,, .				A			
11th ,, .	A	A1	A1				
12th ,, .						A1	A(3)

NOTE.—Numbers in brackets indicate the thickness of crowd.
D indicates death of hopper.

Experiment 8

Previous history.—The hoppers in this experiment were taken from two hatchings.

Reference No. of the parents, P 54 and P 57.

The parents were collected at Pasni on 8th August 1932 and received at Lyallpur on 17th August 1932. They were of '*Solitary*' phase and were reared in pairs.

The eggs were laid on 31st August 1932 and hatched on 14th September 1932. The hatchings of P54 and P57 consisted of sixty-three and sixty-four green hoppers respectively. The hoppers thus had a cent per cent '*solitaria*' tendency.

Associates.—Each *Schistocerca gregaria* hopper had fifteen adult common grasshoppers (predominatingly *Chrotogonus* sp.) as its associates. Fresh catches from the fields replaced the dead associates.

Conditions.—Each hopper of the Desert Locust along with its fifteen associates was reared in a cubical cage measuring 6 in. × 6 in. × 6 in. and with four sides of wire gauze, the botton of wood and the top of a sliding glass sheet. Fresh cotton leaves were dropped into the cage four times a day. The room temperature during the period was:—

Highest maximum—35·0°C.	Highest minimum—32·0°C.
Lowest maximum—21·6°C.	Lowest minimum—16·6°C.
Average maximum—26·08C.	Average minimum—22·03°C.

Remarks.—The experiment confirms the results of the previous experiments. All the hoppers exhibited intense *gregaria* coloration in the fourth instar. The intensity of coloration we are inclined to attribute to the density of the crowd of the associates (15). The progress of the black pigmentation from instar to instar is given in Table XII and the life cycle of these hoppers is given in Table XIII.

TABLE XII

Progress of black pigmentation in hoppers in experiment 8

Reference No.	I stage	II stage	III stage	IV stage	V stage	Remarks
P54-57/C1 . .	Green type . .	Intermediate near green type	.....	.....	...	Killed and eaten in the II stage by the other hoppers
P54-57/C2 . .	Ditto . .	Almost green type	.....	.....	.....	Died in II stage
P54-57/C3 . .	Ditto . .	Intermediate near green type	Intermediate very near green type	Intense black type	.....	Died in IV stage
P54-57/C4 . .	Ditto . .	Black type . .	Intense black type	Ditto .	.....	Died in IV stage
P54-57/C5 . .	Ditto . .	Intermediate near green type	Intermediate very near black type	Ditto .	Intense black type.	
P54-57/C6 . .	Ditto . .	Intermediate near green type	Ditto .	Ditto .	Ditto .	
P54-57/Cont. 1 .	Ditto . .	Geren type . .	Green type . .	Green type . .	Green type . .	
P54-57/Cont. 2 .	Ditto	Ditto . .	Ditto . . .	Ditto . .	Ditto . .	Control hoppers reared singly
P54-57/Cont. 3 .	Ditto . .	Ditto .	Ditto . . .	Ditto .	Ditto . .	Control hoppers reared singly

TABLE XIII

Life history of hoppers in experiment 8

Date (1932)	Daily temperature (°C.)		Experimental					
	Max.	Min.	P54-57 C1	P54-57 C2	P54-57 C3	P54-57 C4	P54-57 C5	P54-57 C6
14th Sept. . .	34·5	31	I	I	I	I	I	I
15th ,, . .	35	32						
16th ,, . .	34·5	32						
17th ,, . .	35	30						
18th ,, . .	34	30						
19th ,, . .	35	30		II			II	II
20th ,, . .	34	29·2	II		II	II		
21st ,, . .	33·5	29						
22nd ,, . .	33·5	29						
23rd ,, . .	33	29						III
24th ,, . .	33	28·5	IID		III	III	III	
26th ,, . .	32·5	28·4						
27th ,, . .	32·5	28						
28th ,, .	32·5	28						
29th ,, . .	32·5	28·5		IID				
30th ,, . .	33	29						
1st Oct. . .	33	29						
2nd ,, . .	33	29						
3rd ,, . .	32·2	28						
4th ,, . .	32·5	28·5						
5th ,, . .	31·5	27·5						
6th ,, . .	31·5	27						
7th ,, . .	31·5	27						
8th ,, . .	31·5	26·5						
9th ,, . .	30·5	26						
10th ,, . .	30	26						IV
11th ,, . .	29·5	25·5				IV	IV	
12th ,, . .	29·5	25·5						
13th ,, . .	30·5	25·5						
14th ,, . .	30	25						
15th ,, . .	30	25				Died		
16th ,, . .	30	25						
17th ,, . .	30·5	25						
18th ,, . .	31·2	24						
19th ,, . .	31	24						
20th ,, . .	29	23						

TABLE XIII—*contd.*

Life-history of hoppers in experiment 8—contd.

Date (1932)	Daily temperature (C.)		Experimental					
	Max.	Min.	P54-57 C1	P54-57 C2	P54-57 C3	P54-57 C4	P54-57 C5	P54-57 C6
21st Oct. . .	29	23						
22nd ,, . .	29	23						
23rd ,, . .	29	23						
24th ,, . .	29	23						
25th ,, . .	28	22						
26th ,, . .	28	22						
27th ,, . .	28	22						
28th ,, . .	28	21						
29th ,, . .	28	20						
30th ,, . .	21	20						
31st ,, . .	21	20·5			IV			
1st Nov. . .	22·7	20·5			stopped			
2nd ,, . .	21	18·3						
3rd ,, . .	23·8	18·8						
4th ,, . .	23·3	18·8						
5th ,, . .	23·3	18·8						
6th ,, . .	23·3	17·7						
7th ,, . .	23·3	18·3						
8th ,, . .	23·3	17·7						
9th ,, . .	23·3	17·7						
10th ,, . .	22·7	17·7						
11th ,, . .	22·7	17·7						
12th ,, . .	22·7	17·7					V	
13th ,, . .	22·7	17·7					stopped	
14th ,, . .	22·7	18·3						
15th ,, . .	23·3	17·7						
16th ,, . .	22·7	17·7						
17th ,, . .	22·7	17·2						
18th ,, . .	21·6	17·2						
19th ,, . .	22·2	17·3						
20th ,, . .	22·2	16·6						
21st ,, . .	22·2	16·6						V

Experiment 9

The hoppers employed in this experiment were of the '*solitaria*' type.

Associates.—The adults of house cricket (*Gryllus domesticus*) were the associates selected for this experiment. Their strength was maintained between fifteen and twenty in each cage.

Conditions.—The cages employed measured 6 in. × 6 in. × 6 in. and had three of the sides and the top of wire gauze. The bottom was soil and the fourth side was the glass door. Fresh leaves were supplied as food.

The experiment was conducted during the summer of 1931.

Remarks.—The hoppers in this experiment had about the same degree of '*solitaria*' tendency as in experiment (4) and were reared in cages of similar dimensions. The number of associates was about the same as in experiment (8) and dicidedly more than in any of the experiments (5), (6) and (7). The 'associates' in this case were the adults of *G. domesticus* as against *P. pictus* and *Chrotogonus* spp. in other experiments. All other conditions in this experiment were practically the same as in experiments (4), (5), (6), (7) and (8), but the strength of associates was higher. One would have expected a better development of the black pigmentation, but, on the contrary, all the hoppers, instead of developing black pigmentation, continued to maintain their '*solitaria*' coloration. The absence of the appearance of black pigmentation can only be attributed to the distinctly different behaviour of the associates, *G. domesticus*, which being nocturnal are active at night.

V. Discussion

In experiments (1) to (4), in which isolated '*solitaria*' hoppers of *Schistocerca gregaria* were made to take exercise by walking, the black pattern of the *gregaria* phase appeared in spite of isolation. Again in three of the experiments [Experiments (5), (7) and (8)] where single hoppers of *Schistocerca gregaria* were kept in association with other grasshoppers, typical *gregaria* black patterns appeared. In experiment (6) all the hoppers did not show the typical '*gregaria*' coloration and in experiment (9) all the hoppers behaved as if they were living a life of absolute isolation, that is, none of the hoppers showed any signs of *gregaria* coloration. Why this difference ? In the case of experiment (6) we have already hinted that the conditions were near a neutral line, a slight deviation in the environmental conditions on one side or the other resulting in a *solitaria* or *gregaria* coloration.

The explanation for the peculiar results in experiment (9) lies in a different direction. The '*solitaria*' hoppers in this experiment have the largest number of associates, but the associates belong to that class of insects that have habits diagonally opposite to those of the hoppers of the Desert Locust. *Gryllus domesticus* is a nocturnal insect and avoids light. In the cages used in our experiment, it kept itself hidden during the day time, under any lump of soil or below the leaves provided for feeding. Therefore, so far as the question of physical exertion, arising from the frequent contact of the insect under experiment and its associates or from the movement induced through seeing the associates moving about goes, the hoppers of the Desert Locust in association with *G. domesticus*, may, for all practical purposes, be regarded as isolated. Thus, it is not at all surprising that hoppers in such conditions should continue to be unaffected by 'association' and maintain the '*solitaria*' coloration.

This also shows that different insects, as associates, from the view-point of the appearance of '*gregaria*' coloration in the associated hoppers, differ a great deal. The most successful, from this point of view, is the one which is nearest to the hopper of Desert Locust in point of habit and behaviour. It will appear that even *P. pictus* hoppers are not ideal associates, if we bear in mind that in a cage 6 in. × 6 in. × 6 in. the minimum number of *P. pictus* hoppers required is about three whereas even two *Schistocerca gregaria* hoppers bred in such a cage acquire typical '*gregaria*' coloration.

Physical exertion being the main common factor in the experiments detailed above, and in the case of hoppers in a swarm in nature and those reared in laboratory in a crowded condition, where *gregaria* coloration appears, we are justified in concluding that the appearance of black pigmentation can be brought about by muscular activity.

VI. Summary

Two automatic devices, by which isolated hoppers of the Desert Locust were made to take muscular exercise have been described. In the construction of these advantage was taken of: (*a*) positive heliotropism or phototropism and (*b*) negative geotropism of the hoppers.

It has been experimentally proved that an isolated hopper (having '*solitaria*' tendency at the start) develops '*gregaria*' coloration when forced to move through a certain distance each day.

Association with other Orthoptera increases the activity of the hoppers of the Desert Locust with the result that the '*gregaria*' coloration is developed. It has been shown that for this purpose the associates should be akin to the hoppers under experiment in the matter of habit and behaviour. The greater the likeness in this respect, the quicker is the response and the smaller is the number of associates required to bring about the colour change.

Since in the life of hoppers belonging to a swarm or to a crowded laboratory rearing and in the life of the hoppers in the experiments described in this paper, the chief common feature is activity, it is concluded that the black pigment is produced as the result of physical muscular exertions.

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STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

*VI. INFLUENCE OF TEMPERATURE ON THE INTENSITY AND EXTENT OF BLACK PATTERN IN THE DESERT LOCUST HOPPERS BRED CROWDED

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(With Plates XXXVI-XL)

CONTENTS

	PAGE
I. INTRODUCTION	625
II. EXPERIMENTAL TECHNIQUE	626
III. BODY COLORATION OF HOPPERS OF DIFFERENT STAGES	
1. Coloration of typical I stage black hoppers	628
2. Description of the coloration of II stage hoppers bred crowded.	
i. At 24° C.	629
ii. At 33° C.	631
iii. At 40° C.	632
iv. At 44° C.	634
3. Description of the coloration of III stage hoppers bred crowded.	
i. At 24° C.	634
ii. At 33° C.	635
iii. At 40° C.	637
iv. At 44° C.	638

* I. The Micropyle in *Schistocerca gregaria* Forsk. and some other Acridiidae. *Ind. J. Agric. Sci.* **3** : 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6** : 188- 62.

III. Why do Locusts Eat Wool ? A study in the hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6** : 262-67.

IV. Colour Changes and Sexual Behaviour in Desexualised *Schistocerca gregaria* Adults. *Ind. J. Agric. Sci.* **6** : 586-590.

V. Pigmentation and Physical Exertion. *Ind. J. Agric. Sci.* **6** : 591-623.

PAGE

4. Description of the coloration of IV stage hoppers bred crowded.
   i. At 24° C. 638
   ii. A 33° C. 640
   iii. At 40° C. 642
   iv. At 44° C. 643
5. Description of the coloration of V stage hoppers bred crowded.
   i. At 24° C. 643
   ii. At 33° C. 644
   iii. At 40° C. 646
   iv. At 44° C. 648

IV. CONCLUDING REMARKS ON COLOUR VARIATIONS 648
V. DISCUSSION 652
VI. SUMMARY 654
REFERENCES 654

I. Introduction

It is well known that the hoppers of *Schistocerca gregaria* Forsk. exhibit a great range of variation in coloration—from almost pure light green to almost pure black with some yellow patches. Even in the first stage the hoppers exhibit three distinct colour forms. They may have an intense pigmented pattern when they may be termed ' black ', or they may be more or less without pigmentation having light green colour, when they may be termed 'green', or they may have pigmentation less intense and not so extensive, when they may be called ' intermediate '. All through the first instar they retain the pattern and coloration with which they are born. From the same packet of eggs there may appear : (1) a pure black hatching, *i.e.*, all the hoppers are pigmented, (2) a pure green hatching, *i.e.*, all the hoppers are unpigmented, *i.e.*, green, or (3) a mixed hatching, which may consist of (i) black and green, (ii) black, intermediate and green, (iii) black and intermediate, or (iv) green and intermediate. It may be made clear that these colour forms are distinct from the intensification of colour which usually occurs a little time after hatching or after a moult.

Repeated observations, by a number of investigators, on different locusts, have established that the locust hoppers bred isolated or singly, *i.e.*, one hopper in each breeding cage, do not ordinarily develop black pigmented pattern, and, in the absence of pigmentation, the entire coloration of the body is some shade of green. If, on the other hand, more than one hopper are placed together in a small cage, *i.e.*, they are bred crowded, then they develop a black pigment, deposited in a more or less well-marked pattern. The intensity and extent of pigmentation depends, to a certain degree, on the duration of crowding and the density of population. The real cause of this interesting phenomenon is at present not known.

In nature the pigmented forms are characteristic of the swarms and the green forms occur in isolation, although not infrequently green hoppers also occur in the swarms of black hoppers.

During the course of our investigations on the bionomics of *Schistocerca gregaria* Forsk., we have had an opportunity of studying the influence of temperature on the extent of colour pattern and the intensity of pigmentation. The results obtained are very striking and are presented in this paper.

So far no detailed study of the development of pigmentation in different parts of the body of a hopper has been undertaken and vague description in such terms as 'pigmented', 'non-pigmented', 'intermediate', does not convey a precise idea. In this paper, therefore, a full description of the coloration of the hoppers has been given and illustrated by coloured plates. In the descriptions that follow we have employed the usual nomenclature and have introduced certain terms for specific colour patches on various parts of the body (Plate XXXVI).*

We have to acknowledge with thanks the assistance received from the Artist of the Entomological Section, Mr. Siddiqi, in the preparation of these plates.

This investigation was carried out under the scheme of Locust Research financed by the Imperial Council of Agricultural Research. We gratefully acknowledge the generosity of the Council.

II. Experimental technique

The cages employed in these experiments were cylinders of brass wire-gauze (100 meshes to an inch). Each cage was six inches long and two inches in diameter, closed at one end and at the other end it was provided with a split cork through which a cabbage leaf—the food on which hoppers were reared—could be introduced. In all cases young cabbage plants, grown in earthen pots were provided as food and the cages were placed on the growing plant. The pots with the cages were kept in incubators at the required temperature. Thus the hoppers had abundant food always available and it may be assumed that the humidity was always high. There was no special arrangement for ventilation in the incubators, although the space available made the supply of oxygen abundant.

The hoppers were bred at the following constant temperatures :—

24°, 27°, 30°, 33°, 36°, 40° and 44° C.

It may be mentioned that at a temperature below 24° C. and above 44° C., the hoppers were not able to complete their life cycle.

Experiment I

History.—Reference No. L 224.

Both the male and female, the progeny of which was used for this experiment, were obtained from a batch of hoppers consisting of forty black and two intermediate hoppers which had hatched on 11th August 1932. They were bred crowded at room temperature in a wire-gauze cage 9 in.×9 in.×9 in. They acquired wings on 7th September 1932. The females from this batch were fertilized by the males from the same batch. A packet of eggs was laid on 20th October 1932.

* This plate is hypothetical, meant to illustrate the terms used, otherwise striped eyes and black pigment do not go together.

The eggs hatched on 11th November 1932. On the basis of their coloration the hoppers could be classified as :—

black 42, intermediate 4, green 0.

The hoppers selected for this experiment were pigmented and thus came from a stock showing pigmentation for two generations.

Three hoppers were placed in each cage, and bred at the following constant temperatures :—

24° C. . . 2 sets.	27° C. . 1 set.	30° C. . 2 sets.
33° C. . 2 sets.	36° C. . 3 sets.	40° C. . 1 set.

Experiment II

History.—Reference No. P. 74.

Both the male and female, the progeny of which was employed in this experiment, were solitary adults captured from Sonmiani (South Baluchistan) on 31st October 1932. The pair was placed in a wire-gauze cage 9 in. × 9 in. × 9 in. from the date it was captured and kept in the hot house.

The female laid a cluster of eggs on 29th December 1932 and the eggs hatched on 14th January 1933. The hoppers were :—

black 24, intermediate 6, green 0.

The hoppers selected for this experiment were pigmented. The hoppers of this batch although descended from solitary parents were predominantly melanic.

Three hoppers were enclosed in each cage, and one cage was placed at each of the following constant temperatures :—

24°, 27°, 30°, 33°, 36° and 40° C.

Experiment III

History.—Reference No. L 276.

Hoppers were bred crowded in a muslin cage in the open, yielding *gregarious* adults, which acquired wings on 14th November 1932. From the date of acquiring wings two females and one male were placed together in a wire-gauze cage 9 in. × 9 in. × 9 in. and kept in the hot house.

One female laid a cluster of eggs on 1st January 1933. The eggs hatched on 16th January 1933 and produced fifty-five black hoppers. These hoppers again showed marked pigmentation.

In each cage seven hoppers were kept and one cage was placed at each of the following constant temperatures :—

24°, 27°, 30°, 33°, 36° and 40° C.

(For the duration of life-cycle, mortality, etc., see Tables I, II and III).

III. Body coloration of hoppers of different stages

1. Description of the coloration of typical I stage black hoppers (Plate XI, fig. I)

(a) *General colour*

Black predominates, dirty yellowish white appearing at places only. A dorso-lateral yellowish streak starting from behind the compound eyes extends over thorax and abdomen, becoming narrower caudad. On pronotum this stripe extends along the caudal margin and forms a wide comma-shaped whitish area.

(b) *Head*

Frons.—Black, right up to the bases of antennae and beyond.

Clypeus.—Black, except for greyish dirty white along the ventral and lateral margins.

Labrum.—Black, except the notch.

Vertex.—Black on both sides of the dirty white epicranial suture, black colour continues behind the eyes on the genae and is continuous with the black sub-ocular region, leaving a post-ocular patch, which is dirty white suffused with yellowish tinge.

Genae.—Entirely black, sub-ocular genal stripe, therefore, not distinguishable.

Postgenae.—Black.

Compound eyes.—Dark chocolate-brown, excepting a narrow dirty pale yellow stripe along the entire mesal margin. Not striped.

Antennae.—Black, intersegmentally dirty white, basal joints also black.

Mandibles.—Black.

Maxillae. }
Labium. }—Black, palpi intersegmentally dirty white.

(c) *Neck*

Cervical sclerites.—Black.

(d) *Thorax*

Pronotum.—Keel with a white median line and narrow black longitudinal stripes on each side of it, the latter widen towards the caudal portion forming a small metazonal triangle on each side. Rest of the pronotum black except a dirty yellowish white hoop or comma-shaped patch which runs cephalo-caudad, parallel to the keel along the black stripe above mentioned and then curves down along the caudal edge of the pronotum and curves again and runs along the ventral margin.

Meso— and meta-nota.—With a mid-dorsal yellowish white line in continuation of the pronotal keel ; wider black stripes on both sides of it, in continuation

of the similar black stripes on pronotum, bounded by lateral dirty yellow stripes on each side ; entire lateral area below these yellowish stripes black.

Meso-episterna
Meso-epimera
Met episterna
Met-epimera } .—Mostly black with small yellowish spots.

Sterna.—Mostly black with dirty yellow ground colour appearing at places.

(*e*) *Legs*

I and II.—Dirty yellow with numerous smoky dots giving a general smoky appearance ; dots fused at the tibio-femoral joints ; claws black.

III.—Hind femurs : entire anterior as well as posterior muscle-impression areas black ; the black pattern meets dorsally leaving an ill-defined spot of basic colour on the dorsal carina of the femur ; apex of femur black and between this and the black pattern on the body of the femur intervenes a dirty yellow ring.

Tibial spines black.

(*f*) *Abdomen*

Segments-I-X.—Each tergum has a black mid-dorsal stripe with a small ivory coloured mid-dorsal triangle at the caudal margin, which becomes successively fainter and smaller on the hinder segments. Along this black stripe, and running parallel to it on each side, is a dirty white stripe which becomes much narrower on the hinder segments and becomes almost invisible on the last segments. Below this the entire tergal area appears black with the exception of an ivory coloured oblique streak on each segment. In the last three segments the oblique white streak is not distinguishable. Below the oblique streak is, so to say, a continuous black stripe and finally along the margin of the tergum is the yellowish white spiracular stripe.

Telson and *cerci*.—Almost black.

Ventral surface.—Black, with a smoky mid-ventral line.

The above is the description of the coloration of the first stage black-pigmented hoppers which were the starting point of all the experiments described below.

2. DESCRIPTION OF THE COLORATION OF THE SECOND STAGE HOPPERS

i. Coloration of II stage hoppers bred crowded at 24° C. (*Plate XXXVII, fig. 1*)

(*a*) *General colour*

Black and dirty white ; lateral whitish streak along the side extending on thorax and abdomen. A comma-shaped whitish area on the pronotum.

(*b*) *Head*

Frons.—Black predominates : entire frons up to the bases of antennae black, rest dirty white with faint pinkish tinge.

Clypeus.—Black.

Labrum.—Black.

Vertex.—Black triangular areas on each side of a fine yellowish-orange epicranial suture ; black colour is continuous behind eyes and joins the black sub-ocular region, leaving a very small yellowish-orange post-ocular patch.

Genae.—Entirely black, sub-ocular genal stripe, therefore, not distinguishable.

Postgenae.—Black.

Compound eyes.—Uniformly dark reddish-brown ; not striped.

Antennae.—Black, smoky intersegmentally.

Mandibles.—Black.

Maxillae.
Labium. } .—Dull white with black patches, palpi black.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—Keel, anterior 3/4 black, posteriorly white ; narrow dark black stripes on both sides of keel fused towards the cephalic portion and become wider forming triangular areas caudally ; similarly on caudal portion they form the metazonal triangular patches ; lateral aspect of pronotum black, except for a hoop of comma-shaped white patch, wide dorsad and caudad and gradually becoming narrower and whiter ventrad.

Meso- and Meta-nota.—Mid-dorsal whitish line, with black stripe on either side of it, and further down ; below this a dirty white straight stripe and a black stripe ; angle from where the wing pads arise black.

Meso-episterna.—Brown at the margins, brownish black in the middle.

Meso-epimera.—Brownish black.

Met-episterna
Met-epimera } .—Black.

Sterna.—Uniformly brownish black.

(*e*) *Legs*

I.—Dorsally almost all black, but ventrally white ground colour appears at places. Claws black.

II.—Dull white with numerous black dots, black along the joints.

III.—Hind femurs : almost the entire muscle-impression areas on the anterior as well as posterior faces black, with a very faint small patch of yellowish colour on the dorsal carina just about the middle. This black pattern almost fused with a wide black ring round the apex of femur.

Base and apex of tibia black ; spines, tarsi and claws black. A few whitish dots on tarsi.

(*f*) *Abdomen*

Segment I.—Mid-dorsal pigmented stripe black and fairly wide, white triangle at posterior margin reduced. Laterally outline of ear black with eight dots irregularly arranged above it.

Segments II-VIII.—Laterally, below the mid-dorsal black stripe is a narrow straight white stripe running through all the abdominal segments except the last three. The tergal area below this stripe and above the white spiracular stripe looks black on account of the fused masses of black spots. There is an oblique white streak running diagonally on each segment.

Segment IX.—Black laterally, only a white dot at each dorso-lateral corner.

Segment X.—All black.

Terminal portion.—Brownish black.

Ventral surface.—Uniformly brownish black with a whitish mid-ventral streak.

(*ii. Coloration of II stage hoppers bred crowded at 33° C. (Plate XXXVIII, fig. 1*)

(*a*) *General colour*

Dull light yellow tinge predominates, although black pattern also well marked (but not so well developed as in the hoppers bred at 24° C.).

(*b*) *Head*

Frons.—Dull light yellow with fine black dots ; lower portion black, getting fainter above.

Clypeus.—Irregular, blurred black spots ; dorsal portion lighter.

Labrum.—Black, lighter at the emargination.

Vertex.—Black on both sides of the fine white epicranial suture. Black colour creeping behind the eyes. Post-ocular patch brownish orange, extensive, as long as or longer than the compound eye.

Genae.—Sub-ocular genal stripe black, very wide and extends up to the inner margin of the eyes. Area behind this stripe brown.

Postgenae.—Light brown to black.

Compound eyes.—Dark reddish brown, without any stripes : anterior half with a greyish tinge.

Antennae.—Black ; basal one segment whitish. White intersegmentally.

Mandibles.—Black.

Maxillae }
Labium } .—Mixed smoky and white.

(*c*) *Neck*

Cervical sclerites.—Black but lighter in colour.

(*d*) *Thorax*

Pronotum.—Keel white, narrow black stripes along its each side getting wider at the anterior margins forming anterior triangles. Metazonal triangular patches

black, not extensive. Lateral black patches very large, covering more than half of prozona leaving a broad comma-shaped white patch running dorsal and cephalad.

Meso- and *meta-nota.*—Dorsal black stripe on both sides of the white median line ; below this a white straight stripe and then a broad black straight stripe.

Meso-episterna, *Meso-epimera*.—Yellowish white with black dots.

Met-episterna, *Met-epimera*.—Black.

Sterna.—White with large black patches.

(*e*) *Legs*

I and II.—Dull yellowish white, with black dots throughout. Black colour along the joints.

III.—Hind femur : black pattern on the anterior as well as posterior muscle-impression areas, with a small oval gap on the dorsal carina, which runs a little each way on the anterior and posterior aspects. The black pattern not fused with the black ring round the apex of femur.

Both the ends of tibia black ; spines also black.

(*f*) *Abdomen*

Segment I.—A wide dark black stripe dorsally with a white triangular area at posterior margin. Laterally six to seven black dots irregularly arranged.

Remaining segments.—Mid-dorsally, in each segment a wide black stripe with white triangular area at posterior margin; laterally five black dots along the posterior margin ; in front of these a straight black stripe and above it an oblique black streak and a number of (usually four) black dots dorsad to this.

Terminal part of abdomen.—The black pattern distinctly reduced as compared to the hoppers at 24° C.

Ventral surface.—White with large smoky dots.

iii. Coloration of II stage hoppers bred crowded at 40° C. (*Plate XXXIX, fig. 1*)

(*a*) *General colour*

Much lighter than the hoppers at 33° C., but with more of pigment than in the hoppers bred at 44° C. General colour dull whitish with a yellowish tinge.

(*b*) *Head*

Frons, *Clypeus*, *Labrum*.—Uniformly yellowish white without any smoky dots.

Vertex.—Broken smoky areas on both sides of the epicranial suture ; rest very light brown.

Genae.—Sub-ocular genal stripe narrow ; smoky regions in front and behind, dull white.

Postgenae.—Very light brown, almost whitish.

Compound eyes.—Anterior half, light grey ; posterior, dark reddish brown. Not striped.

Antennae.—Very light smoky, whitish intersegmentally.

Mandibles.—White, dentation black, smoky near labium and clypeus.

Maxillae, *Labium*.—White with faint smoky marks on palpi.

(*c*) *Neck*

Cervical sclerites.—Light smoky.

(*d*) *Thorax*

Pronotum.—General colour white, with a yellowish tinge. Keel whitish, very narrow smoky stripes on each side. Lateral patch large brownish, its prozonal portion is mixed smoky and white, and metazonal portion smoky brownish, much reduced. Metazonal triangular patches brownish black. The Comma-shaped area very extensive.

Meso- and meta-nota.—Dorsal brownish black stripe on both sides of white median line, whitish areas below this followed by the brownish black stripe.

Meso-episterna, *Meso-epimera*.—White with two faint black dots.

Met-episterna.—White ; a narrow smoky streak at the posterior margin.

Met-epimera.—White ; a narrow smoky streak at the anterior margin fused with a smoky streak at the posterior margin of met-episternum.

Wing pads.—Smoky.

Sterna.—Uniformly white.

(*e*) *Legs*

I and II.—Dull white ; pinkish marks at the basis of hairs. Smoky marks close to the joints.

III.—Hind femur : a narrow smoky pattern on the anterior muscle-impression area and a similar pattern on the posterior muscle-impression area, leaving a small ring of dull white with yellowish tinge between this pattern and the black colour round the apex of femur. The pattern on the anterior and posterior aspect of the femur fused dorsally except for a broadly oval area on the dorsal carina.

Apex of femur, and base and apex of tibia brownish black. Tarsi brown, claws light black.

(*f*) *Abdomen*

Segment I.—Dorsally light black stripe with a white triangular area at the posterior margin ; 4 smoky dots on the lateral region.

Remaining segments.—Dorsally as in segment I, laterally five brown dots along the posterior margin. In front of these, ventralmost is a straight broken brownish stripe ; above this an oblique brown streak and dorsal to it two to three light black dots.

Terminal portion.—Yellowish white.

Ventral surface.—Dull white without any dots.

**iv. Coloration of II stage hoppers bred crowded at 44° C. (Plate XL, fig. 2)*

Entire body colour almost whitish.

Eyes colourless or light greyish, except dorsal lunar area which is dark. Antennae colourless.

Claws and spines very light reddish brown at tips, but tarsi white. A few scattered dots on the femur, but no trace of the black pattern. Lunar ridge on trocolia, very light brown.

3. DESCRIPTION OF THE COLORATION OF THE THIRD STAGE HOPPERS

i. Coloration of III stage hoppers bred crowded at 24° C. (Plate XXXVII, fig. 2)

(*a*) *General colour*

Black predominating, rest dirty white with an almost imperceptible yellow tinge.

(*b*) *Head*

Frons.—Dorsally dirty to light brown ; lower portion black ; black colour creeping up and gradually fading.

Clypeus.—All black.

Labrum.—Black except near the emargination.

Vertex.—Epicranial suture very faint ; black area on each side, black colour creeping down behind the compound eyes, leaving a small yellowish brown post-ocular patch.

Genae.—Entirely black ; sub-ocular genal stripe not distinguishable.

Postgenae.—Black.

Compound eyes.—Not striped ; dark reddish brown ; anterior margin lighter (dirty white).

Antennae.—Light smoky colour on the basal joint, rest black ; whitish line along the lateral aspect ; terminal joints lighter.

Mandibles.—Black.

Maxillae.—Black ; palpi also black except terminal portion of joints which are lighter.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—Pale yellow dots all over the pronotum. Anterior margin whitish. A black stripe on the keel throughout, except anterior end. The triangular meta-

* The size of the hoppers bred at 44° C. is below the normal. This reduction is more marked in advanced stages. This is clearly seen in Plate XL, figs. 2—5.

zonal patches black fused in the middle. Lateral black patch very large, separated from median line and metazonal patch by a dull comma-shaped band.

Meso- and meta-nota.—Mid-dorsal black stripe in continuation of the black keel of the pronotum. Below this straight white stripe on each side.

Meso-episterna.—Light fawn, with smoky dots.

Meso-epimera.—Dirty white with light yellowish spots.

Met-episterna
Met-epimera } .—Black.

Wing pads.—Entirely black.

Sterna.—Smoky ; presternum black.

(*e*) *Legs*

I and II.—Coxae and trochanters smoky ventrally and at some other places. Femur brown to black dorsally towards apex. Tibia, head and apex black, anterior aspect black. Tarsi black, less black in II pair.

III.—Hind femur : anterior muscle-impression area entirely black except near base and apex ; its distal end extends dorsally and posteriorly with an ill-defined gap between, and ends just above the postero-ventral femoral carina. Apex of femur black.

Head and apex of tibia black. Spines black. Tarsi dorsally black.

(*f*) *Abdomen*

Segment I.—Black median stripe wide, with white triangular area. Laterally two rows of irregular spots on dirty white ground colour.

Segments II-VIII.—Dorsally as in segment I, laterally brown to black stripe just above the white spiracular stripe, then well-marked oblique black streak, and two rows of black irregular spots above this.

Segment IX.—Laterally dark except a very small dirty white patch.

Segment X.—Entirely black.

Telson.—Smoky with black spots.

Ventral surface.—Brownish to black areas on both sides of a whitish median streak.

ii. Coloration of III stage hoppers bred crowded at 33° C. (Plate XXXVIII, fig. 2)

(*a*) *General colour*

Dull white with a pinkish and yellowish tinge.

(*b*) *Head*

Frons.—Pink, dirty white at places ; two black dots at the lower end of each frontal carina.

Clypeus.—Dull white with a greyish tinge, black along ventral margin.

Labrum.—Black, with dull white spots at places.

Vertex.—Black areas on both sides of epicranial suture which is a fine yellowish orange line. Black colour continued down behind the eye, leaving an orange-yellow post-ocular patch.

Genae.—Dull white in front of the sub-ocular genal stripe, which is a wide black streak creeping up behind the eye, but here it is blurred black.

Post-genae.—Dull white.

Compound eye.—Striped ; three reddish brown stripes, almost equally wide, with whitish interstripes.

Antennae.—Black, basal segments white, and whitish along the lateral aspect.

Mandibles.—Anteriorly black, laterally brown, posteriorly smoky.

Maxillae, *Labium*.—Dull white, palpi black and white.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Basic colour dull white with a pinkish tinge and pale yellow dots. Light pink colour along the sides of keel, which has a fine white line with a narrow black stripe along each side. These black stripes become broader anteriorly and posteriorly ending in triangular metazonal patches. Lateral black pattern, lighter in the prozonal region, dark black in metazonal region. Intervening comma-shaped patch broad and white with a pinkish tinge particularly in the anterior half of it.

Meso- and meta-nota.—Mid-dorsal stripe black and in continuation of the black keel of the pronotum ; below this straight white stripes with a pinkish tinge.

Meso-episterna, *Meso-epimera*.—Light yellow.

Met-episterna.—Dorsal half black, ventral dull white with a vertical black line in the middle.

Met-epimera.—Black.

Wing pads.—Black.

Sterna.—White with black dots.

(*e*) *Legs*

I and II.—Dull white with dark-brown dots. Claws light black. Femoro-tibial joints brownish, the brown colour going each way about 1/8th length of femur and tibia. Spines and claws black ; spurs black with brown ends.

III.—Hind femur ; black pattern on the anterior muscle-impression area covering dorsal 2/3 of entire surface except (i) about 1/4 of its proximal end between the antero-dorsal carina and the muscle-impression area and (ii) a small gap on the dorsal margin of distal end. The two extensions of the pattern on either side of the gap grow wider as they are continued to the posterior side and end on the posterior muscle-impression.

(*f*) *Abdomen*

Segment I.—A wide black dorsal median stripe, with a white triangle at the posterior margin, the vertex of which is continued anteriorly in short distances as a fine white line. Laterally three dorso-ventral rows of dots ; three dots in the posterior row, two in the middle and one to two in the anterior.

Segments II-VIII.—Dorsal stripe as in segment I. Laterally six dots along posterior margin ; dorsal two dots almost touching the dorsal stripe. In front of the ventralmost dot is a straight black stripe, above it the oblique streak and over this three-four dots. Basic colour dull white.

Segment IX.—Laterally only a black line along its posterior margin.

Segment X.—Laterally scattered black spots ; terminal portion dull white.

Ventral surface.—White with black dots.

iii. Coloration of III stage hoppers bred crowded at 40° C. (*Plate XXXIX, fig. 2*)

(*a*) *General colour*

White with a slight yellowish tinge.

(*b*) *Head*

Frons, *Clypeus*, *Labrum*.—Uniformly pale yellowish.

Vertex.—Narrow black streaks on each side of the epicranial suture which is light brown ; post-ocular patch extensive orange-yellow.

Genae.—Whitish yellow in front and light orange-yellow behind the narrow smoky sub-ocular genal stripe.

Postgenae.—Very light brown, almost whitish in places.

Compound eyes.—Striped ; four chocolate-brown stripes visible with whitish grey inter-stripes.

Antennae.—Smoky in the middle, whitish towards the distal and basal ends.

Mandibles.—Exposed parts whitish, dentation black.

Maxillae, *Labium*.—White, faint black dots on palpi.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Pale yellow ; keel whitish behind the hind sulcus. Reduced black pattern in the prozonal region ; blurred brownish-black patch laterally in metazonal region. The metazonal patches, triangular in form on each side of median line, are brownish black with whitish dots.

Meso- and meta-nota.—Mid-dorsal black stripe in continuation of the black keel of the pronotum. Below this there is a straight white stripe.

Meso-episterna
Meso-epimera } .—Uniformly pale yellow.

Met-episterna.—Pale yellow ; blurred black spots along posterior margin.

Met-epimera.—Pale yellow ; blurred black spots along anterior margin.

Wing pads.—Black dots on the base as well as on the pad.

Sterna.—Pale yellow without any dots.

(*e*) *Legs*

I and II.—Dull white without black markings.

III.—Hind femur ; black pattern on the anterior muscle-impression area not wide and diffused. Its dorsal margin is separated from the antero-dorsal carina by a wide streak of basic colour except near the distal end where two narrow extensions of the pattern run dorsally, enclose a wide gap of ground colour and end on the postero-dorsal carina.

(*f*) *Abdomen*

Segment I.—Tergum has dorsally a light black narrow stripe with a large white triangular area at the posterior margin ; four to five black dots on each lateral aspect.

Segments II-VIII.—Tergum dorsally as in segment I. Laterally a row of five black dots along posterior margin. Opposite to the lowest dot is a smoky straight stripe, above this an oblique smoky streak and over this three smoky dots parallel to the oblique streak.

Segments IX-X.—Tergum laterally with only a row of black dots, fused into a line along the posterior margin.

Genital appendages.—Light yellow.

Ventral surface.—Yellow with faint brownish dots.

iv. Coloration of III stage hoppers bred crowded at 44° C. (*Plate XL, fig. 3*)

Body colour whitish with a pale yellowish tinge.

Eyes whitish anteriorly, darker along the posterior margin. Lunar area of the eye dark. Antennae white.

Frons and mouth-parts white, post-ocular region pale yellow. Legs whitish, dull yellow on the anterior surface of hind femur.

Abdomen white with a pale yellow tinge ; brownish dots on the lateral aspect.

4. DESCRIPTION OF THE COLORATION OF THE FOURTH STAGE HOPPERS

i. Coloration of IV stage hoppers bred crowded at 24° C. (*Plate XXXVII, fig. 3*)

(*a*) *General colour*

Black predominating ; ground colour dirty yellow with a mixed green and pinkish tinge.

(*b*) *Head*

Frons.—Dull yellow ; ventral about 1/8th black, black colour getting fainter upwards. Numerous black dots along the carinae.

Clypeus.—Black.

Labrum.—Black, notch lighter.

Vertex.—Epicranial suture not distinct. Entire area behind eyes black, joining the sub-ocular black area, except a very small orange-red post-ocular patch.

Genae.—Black ; sub-ocular genal stripe, therefore, not distinguishable.

Post-genae.—Black.

Compound eyes.—Not striped ; uniformly dark reddish brown.

Antennae.—Dark black except the basal two segments which are dirty yellow. Lateral aspect of antennae lighter.

Mandibles.—Black.

Maxillae }
Labium } .—Black. Palpi intersegmentally whitish.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—Entire pronotum, except a reduced 'comma-shaped' patch, is black with yellow specks and spots. This patch intervenes between the triangular metazonal patch and the rest of black prozona. White along the ventral margin as well as lower part of anterior and posterior margins. Keel white behind the posterior sulcus, black in front of it.

Meso-episterna.—Dull deep brown ; irregularly black along the anterior margin.

Meso-epimera.—Dull deep brown ; black along dorsal and posterior margin.

Met-episterna.—Black.

Met-epimera.—Black.

Wing pads.—Base black, black lines along veins.

Sterna.—Numerous irregular black patches on a dirty brown ground colour.

(*e*) *Legs*

I and II.—Dull yellow, black ventrally. Femur with dorsal distal half black. Tibia black at both the ends ; spines black. Tarsi dorsally black, ventrally brownish.

III.—Hind femur : the anterior muscle-impression area entirely black with two dark wide extensions at its distal end enclosing a very narrow area of basic colour. The two arms get wider on the muscle impression area of the posterior aspect and end on the postero-ventral carina. Distal end of femur black leaving a ring of basic colour between.

Tibia black at both ends. Spurs black ; tarsi brownish black with black spots ; claws black.

(*f*) *Abdomen*

Segment I.—Tergum covered under wing pads. Dorsal black stripe wide with a white triangular area at the posterior end. Laterally three dots along the posterior margin, a few irregular black dots in front.

Segments II-VIII.—Mid-dorsal stripe as in segment I ; laterally along ventral margin a straight wide black stripe ; above this an oblique black streak over this three to four black dots parallel to the oblique streak ; two dots above this towards posterior margin.

Segment IX.—Tergum dorsally as above, laterally just a line of white colour below the dorsal stripe. Below this all black except a few whitish dots.

Segment X.—Tergum all black. Cerci smoky.

Ventral surface.—Numerous black dots on a dirty brown basic colour, except a yellow streak running mid-ventrally along the length of abdomen.

ii. Coloration of IV stage hoppers bred crowded at 33° C. (Plate XXXVIII, fig. 3)

(*a*) *General colour*

Light yellow with a greenish tinge predominates.

(*b*) *Head*

Frons.—Bright yellow ; a few brown dots near the ventral margin.

Clypeus.—Dull yellow ; dark brown area in the middle and along the lateral margins.

Labrum.—Almost all black.

Vertex.—A triangular black area on each side of epicranial suture. Each side of triangle continued as a black line behind eye and joining the black sub-ocular genal stripe. Post-ocular region brownish orange, extensive.

Genae.—Sub-ocular genal stripe black with a small yellow triangular area in front.

Post-genae.—Black.

Compound eyes.—Striped, with three dark reddish brown stripes. Posterior stripe widest covering half of eye ; the other two stripes are narrow streaks.

Antennae.—Smoky black excepting basal two segments. Intersegmentally faintly whitish.

Mandibles.—All black, except a small yellow spot at base.

Maxillae } —Pale yellow ; palpi black, only at places with whitish basic
Labium } colour.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Greenish yellow with brighter spots, white along posterior and ventral margins. Keel straight from posterior margin to hind sulcus ; slightly convex in front of it. Black in front of hind sulcus, yellow behind it. Lateral black pattern faint in the prozonal region dark in the metazonal region, bent forward at the top. Metazonal triangular patches black with yellow dots.

Meso-episterna.—Uniformly yellow with a greenish tinge. Dorsal margin smoky.

Meso-epimera.—Yellow with a greenish tinge and brighter dots. Dorsal margin smoky. Pleural suture smoky.

Met-episterna.—Greenish yellow ; black patches mixed irregularly ; intenser black along posterior margin.

Met-epimera.—All black, blackness creeping up to the base of the wing pads.

Wing pads.—Base black ; black dots and lines on wing veins.

Sterna.—Yellow with black dots.

(*e*) *Legs*

I and II.—Greenish yellow, light black colour on femur and tibia at the tibio-femoral joint, also at the tibio-tarsal joint. Tarsi dark brown, claws and spines black.

III.—Hind femur : the black pattern on the anterior muscle-impression area wide with two dark black extensions at its distal end going dorsally and ending in the middle of the posterior muscle-impression area. The mesal arm is darker and broader than the other.

Both the ends of tibia light black. Spurs black. Tarsi greenish ; claws deep brown.

(*f*) *Abdomen*

Segment I.—Tergum covered by wing pads. Dorsal stripe faint blackish with a white patch at posterior margin. Four black dots along the posterior margin dorso-ventrally.

Segments II-VIII.—A wide black mid-dorsal stripe with white triangular patch near posterior margin. Five broad black spots along posterior margin ; the ventralmost fused with a fairly wide straight black stripe, the 2nd is fused with an oblique black streak. Against the 3rd dot, there are three dots parallel to the oblique streak, one dot opposite to 4th ; the 5th fused with the dorsal stripe.

Segment IX.—The lateral pattern consists of one wide black streak.

Segment X.—Black.

Telson.—Greenish yellow.

Ventral surface.—Greenish yellow with smoky dots.

iii. Coloration of IV stage hoppers bred crowded at 40° C. (Plate XXXIX, fig. 3)

(*a*) *General colour*

Light yellow.

(*b*) *Head*

Frons, *Clypeus*, *Labrum*.—Whitish yellow (without any dots).

Vertex.—Smoky along both sides of the epicranial suture. Post-ocular patch brownish orange.

Genae.—Pale yellow in front and light orange-yellow behind the sub-ocular genal stripe, which is narrow and slanting.

Post-genae.—Very light yellow (almost whitish).

Compound eyes.—Striped ; four narrow, light chocolate-brown stripes with almost whitish broad interstripes.

Antennae.—Smoky in the middle, whitish at the distal and basal ends.

Mandibles.—Exposed parts yellow, dentation black.

Maxillae, *Labium*.—Pale yellow.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Bright yellow, white along the posterior margin. A very fine white streak on keel. On the lateral aspect of prozona a faint smoky patch with yellow dots. The metazonal triangular patches much reduced ; black with bright yellow dots.

Meso-episterna, *Meso-epimera*.—White with a yellow tinge. Pleural suture and dorsal margin smoky.

Met-episterna.—Yellow ; black at places, particularly toward the anterior margin.

Wing pads.—A black dot at base, rest pale yellow, with remnants of black lines on veins.

Sterna.—Whitish with a few fine dots.

(*e*) *Legs*

I and II.—Uniformly pale yellow, except tarsi which are laterally faint smoky. Claws and spines brown.

III.—Hind femur : black pattern on the anterior aspect covering only the dorsal 2/3 of the muscle-impression area and separated from the antero-dorsal carina by a wide streak of basic colour, except the two narrow smoky extensions at its distal end enclosing a wide gap of basic colour. The pattern is extremely

faint and narrow (as if almost broken) at the ventral end of the gap. The two extensions are continued to the posterior aspect, but do not cover the posterior muscle-impression area and end a little below the postero-dorsal carina.

Spurs smoky ; claws light brown. Spines black tipped ; black dots on terminal end of femur.

(*f*) *Abdomen*

Segments I-X.—Tergum of segment I covered by wing pads ; dorsal black stripe irregular in segment II and III, in others it is present as elongated patch with a white triangular area at the posterior margin. In the lateral aspect there are five to six smoky dots along posterior margin. A narrow straight smoky stripe opposite to the ventralmost, above this an oblique streak and over this five to six black dots.

Telson.—Yellow.

Ventral surface.—Yellow with a few faint smoky dots.

iv. Coloration of IV stage hopper bread crowded at 44° C. (Plate XL, fig. 4)

General colour white.

Eyes white with two very fine, light brown stripes ; lunar area dark. Sub-ocular genal stripe extremely faint smoky ; light brownish along the posterior margin in the post-ocular region. Rest of face and head white. Antennae white.

Pronotum almost white.

Legs almost white.

Abdomen whitish with grey colour at places. Fine brownish dots along posterior margin of some of the abdominal segments.

5. DESCRIPTION OF THE COLORATION OF THE FIFTH STAGE HOPPERS

i. Coloration of the V stage hoppers bred crowded at 24° C. (Plate XXXVII, fig. 4)

(*a*) *General colour*

Black predominating, with light yellow, pinkish and greenish tinge at places.

(*b*) *Head*

Frons, *Clypeus*, *Labrum*.—All jet black.

Vertex.—All black, except a small brownish portion dorsad of the base of antennae. Post-ocular patch brownish, very much reduced, and concealed under pronotum. Epicranial suture very faint.

Genae.—All jet black, sub-ocular genal stripe not distinguishable.

Compound eyes.—Dark, reddish-brown. No stripes.

Mandibles, *Maxillae*, *Labium*.—Black.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—Entire pronotum jet black ; dotted with very small greenish yellow spots. Sulci along the dorsal portion have pinkish tinge. No separate metazonal patches.

Meso-episterna.—Jet black, with three very small insignificant yellowish dots.

Meso-epimera.—Black with larger and more prominent yellow dots.

Met-episterna.—Jet black, two small yellow dots.

Met-epimera.—Jet black, three small yellow dots.

Wing pads.—Base black, all veins black, rest of wing greenish yellow.

Sterna.—Brownish black.

(*e*) *Legs*

I and II.—Pinkish brown with black colour over the greater area.

III.—Hind femur : anterior muscle-impression area completely black and fused with the black pattern on the posterior muscle-impression with the exception of the proximal end and a narrow gap of ground colour near the distal end. The posterior black pattern ends on the postero-ventral femoral carina.

Apex and head of tibia jet black, rest dirty orange-brown. Tips of spines, spurs, and dorsal tarsi, black.

(*f*) *Abdomen*

Segment I.—Tergum covered under wing pad, brownish.

Segments II-VIII.—Mid-dorsal stripe very wide, and black with posterior whitish triangular area. Above the white spiracular stripe is a broad black stripe. Above this an oblique streak which is most prominent and, going up along the interior margin, meets the dorsal stripe. Over this two broken streaks. Very small area of the abdomen appears greenish yellow and even this is suffused with the brown.

Segments IX and X.—Tergum dark black.

Telson.—Yellowish brown.

Ventral surface.—Brownish black with a lighter mid-ventral line.

ii. Coloration of V stage hoppers bred crowded at 33° C. (Plate XXXVIII, fig. 4)

(*a*) *General colour*

Brownish yellow.

(*b*) *Head*

Frons.—Brownish yellow, darker along the carinae. Few fine black dots close to the ventral margin. Two dots near the vertex.

Clypeus.—Dark brownish yellow ; darker near ventral margin ; black oblique line coming from each dorso-lateral corner.

Labrum.—Black ; a light brown streak exactly in the middle ; lighter along lateral margin, light brown near the notch.

Vertex.—Black on either side of a very faint epicranial suture. The black colour continued down behind the eyes to the sub-ocular genal stripe. Post-ocular area orange.

Genae.—Sub-ocular genal stripe black. In front of it and behind it a yellow area with black dots.

Post-genae.—Pale yellow, with blurred black colour along anterior margin.

Compound eyes.—Five reddish brown stripes. Interstripal area between anterior three stripes whitish, between others light reddish brown, so that stripes are just distinguishable.

Antennae.—Black ; basal two segments and lateral aspect whitish.

Mandibles.—Anterior and posterior aspect black, laterally black with a light yellowish area.

Maxillae } .—Dull white, palpi black, intersegmentally yellowish. Tip of
Labium } galea black.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—White along ventral and posterior margin. Keel black on the prozonal region and yellow in the metazonal region. Lateral black pattern large, well marked in the metazonal region, blackish brown with yellow spots in the prozonal region. Between the black triangular metazonal patch and the lateral pattern is a brown to yellow comma-shaped band.

Meso-episterna.—Uniformly brown.

Meso-epimera.—Brown with brighter dots.

Met-episterna.—Black, irregular whitish patches at places.

Met-epimera.—Uniformly black.

Wing pads.—Base all black ; black continuous lines on the anterior veins ; black dots on posterior veins.

Sterna.—Yellow with fine smoky dots.

(*e*) *Legs*

I and II.—Brown, a black spot at the apex posteriorly, black dots near the femoral end of tibia. Tarsi light brown ; claws blackish.

III.—Hind femur : a wide black stripe on the anterior muscle-impression area leaving only a narrow line of ground colour above the antero-ventral femoral carina.

Its two dorsal extensions on the distal end are brownish black, and enclose a gap of ground colour almost as wide as the thickness of each extension.

On the posterior aspect the mesal extension is broader and, crossing the posterior muscle-impression area, ends a little above the postero-ventral carina. Distal arm ends a little below the postero-dorsal carina. Apex of femur black.

The head of tibia black ; spines brownish. Spurs dark brown. Tarsi and claws brown.

(f) Abdomen

Segment I.—Tergum covered under wing-pads, faint markings.

Segments II-VIII.—Mid-dorsal stripe broad, five dots along posterior margin, the lowermost fused with the ventral straight stripe. In front of the 2nd a broken oblique streak. In front of the 3rd and 4th dots, a number of dots irregularly situated. The 5th dot fused with the dorsal stripe.

Segments IX-X.—Tergum dark brown.

Ventral surface.—Yellow, with black dots.

(iii. Coloration of V stage hoppers bred crowded at 40° C. (Plate XXXIX, fig. 4)

(a) General colour

Bright yellow, with a slight greenish tinge ; very little of black pigmentation.

(b) Head

Frons.—Bright yellow, slightly greenish along frontal carinae, no black dots.

Clypeus.—Dorsal half bright yellow, ventral dull white with a yellowish tinge.

Labrum.—Yellow in the middle, dull white along the lateral margins.

Vertex.—Blackish triangular areas on each side of the epicranial suture. A yellow line between compound eye and the above area. Post-ocular patch orange-yellow. The black coloration does not fuse with sub-ocular genal stripe.

Genae.—Yellow, both in front of and behind the black sub-ocular genal stripe, which tapers downwards and is not continued behind the eyes.

Post-genae.—Yellow.

Compound eyes.—Five-six light chocolate-brown stripes with whitish interstripes.

Antennae.—Basal and distal segments whitish or light brown ; middle smoky; whitish along the ventral side.

Mandibles.—Yellow ; anterior margin black.

Maxillae, *Labium*.—Pale yellow ; tips of palpi smoky.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Yellow with brighter specks, white along the anterior, ventral and posterior margins. Keel yellow in front and white behind the hind sulcus. Lateral black pattern absent, only faint smoky colour at the base of posterior sulcus. Smoky metazonal triangular patches with yellow dots on each side of the median line, narrower and darker in front, wider and fainter posteriorly.

Meso-episterna.—Yellow without any black dots.

Meso-epimera.—Uniformly yellow, whitish near the posterior margin.

Met-episterna.—Yellow, a black streak along the posterior margin.

Met-epimera.—Yellow, a black streak along the anterior margin.

Wing pads.—Black dots at the base of wings. Dots on dorsal longitudinal veins, lines on lower longitudinal veins.

Sterna.—Uniformly yellow.

(*e*) *Legs*

I and II.—Femur and tibia yellow, tarsi light brown ; claws and spines tipped with brown.

III.—Hind femur : on the anterior aspect black colour only on the dorsal 2/3 of the muscle-impression area, the remaining 1/3, above and along the antero-ventral femoral carina yellow. The black extensions at the distal end of muscle-impression area light, *i.e.*, smoky and enclose a wide patch of ground colour. These two extensions go up, cross the dorsal carina and end on the posterior aspect just below the postero-dorsal femoral carina. The distal arm is very faint on the posterior aspect.

(*f*) *Abdomen*

Segment I.—Tergum covered under wing-pads, uniformly yellow.

Segment II.—Dorsal black stripe on tergum absent. A row of four black dots dorso-ventrally on each lateral aspect.

Segments III-VIII.—On tergum, dorsal stripe blurred black, interrupted by a white patch at the posterior margin. Laterally five elongate black dots along the posterior margin. The ventralmost united with the straight black stripe, the 2nd opposite to a faint brown oblique streak usually cut up. Opposite to the 3rd and 4th are three or four dots irregularly arranged.

Segment IX.—Mid-dorsal stripe and lowermost straight stripe faint smoky, rest yellow.

Segment X
Telson }.—Yellow.

Ventral surface.—Yellow.

iv. Coloration of the V stage hoppers bred crowded at 44° C. (*Plate XL, fig. 5*)

General colour white, pale yellow tinge at some places. Eyes with three light brown stripes, interstripes white. Extremely faint, smoky sub-ocular genal stripe visible. Light brownish yellow along the posterior margin of the post-ocular region. Remaining face and antennae white.

Pronotum white behind the hind sulcus, pale yellow tinge in front of it.

Legs white, light brown fish-bone area on the anterior aspect of hind femur.

Abdomen white with a very light yellowish tinge. Faint smoky dots along the posterior margin of each abdominal segment.

IV. Concluding remarks on colour variations

From the detailed description of the hoppers of the different stages, bred crowded, at various constant temperatures, *viz.*, 24° C., 33° C., 40° C. and 44° C., it is possible to summarise the following general conclusions :—

(*a*) *General colour*

At 24° C. the black colour is most intense and the black pattern most extensive, *i.e.*, the black predominates. At 33° C. the pigmentation is generally reduced and the general body colour is light yellowish in II and III stages, and greenish or greenish yellow in IV and V stages. The black pattern is present at 40° C. although much reduced. At 44° C. the black pattern is almost absent. The colour is uniformly dull white to pale yellow and there are no black markings except faint smoky design in some cases. Thus with successive rise in temperature the black pigmentation gets reduced and is finally eliminated.

(*b*) *Head*

At 24° C. the entire front, clypeus, labium and genae arejet black, therefore the sub-ocular genal stripe is not distinguishable. At 33° C. the black colour is confined to the lower part of the head—the labrum is entirely black, while clypeus may be only black or spotted ; the frons has a uniform pinkish colour with fine black dots on the carinae. The black sub-ocular genal stripe is broad, creeping up along the margin of the compound eyes. The rest of the gena has the ground colour of the body. At 40° C. the entire face including frons, clypeus and labrum, has the uniform dull white colour in the II and III stages and yellow or greenish yellow in IV and V stages without any black markings or dots. The sub-ocular genal stripe is very narrow and smoky or has a bluish tinge. At 44° C. no black pigment is present.

Vertex and post-ocular region.—At 24° C. the epicranial suture is faint or indistinct and the vertex and post-ocular region are black, except a very reduced post-ocular brownish orange patch. At 33° C. there are well-marked triangular areas on each side of the epicranial suture. The lower angle of the triangles is continued downwards as a narrow streak behind the compound eyes joining the

sub-ocular black portion. The post-ocular patch is extensive and orange-yellow in colour. At 40° C. broken triangular areas are present on each side of the epicranial suture and are not continued downward, nor fused with the sub-ocular genal stripe. The post-ocular patch is brownish in II stage and yellowish orange in the remaining stages. The rest of the post-ocular region has the ground colour of the body.

Compound eyes.—At 24° C. the eyes are not striped and have a uniform dark reddish brown coloration. At 33° C. the hoppers of II stage retain the uniform dark reddish brown coloration, but those of the later stages have striped eyes. The stripes are reddish brown with interstripes. There are three or four such stripes in III, four or five in IV and five or six stripes in V stage. At 40° C. the eyes are striped in all the stages. In II stage the anterior half is light grey and the posterior is dark brown. This fact is of interest because the striped eyes are regarded as a character of the *solitary* hopper. In these experiments the crowded hoppers have attained the striped conditions.

The situation may be explained thus. The eyes are essentially striped in *Schistocerca gregaria*, but these stripes are concealed by superimposed pigmentation and when that is removed through any cause, the stripes become evident.

Antennae.—At 24° C. and 33° C. the antennae are black excepting the basal two segments which are lighter, and a lighter line along the lateral aspect of the antennae. At 40° C. the antennae are smoky in the middle and whitish at the base and tip.

(*c*) *Cervex*

The cervical sclerites are black at 24° C. and 33° C., but lighter, *i.e.*, smoky at 40° C.

(*d*) *Thorax*

Pronotum.—At 24° C. in II, III and IV instars there are prominent black stripes on each side of the median line, which are usually fused with each other in the prozonal region. They widen up anteriorly to form small black triangular areas, and also posteriorly to form triangular metazonal patches. In the lateral region there is a large black pattern covering the whole of prozona and greater portion of the metazona, leaving only a comma-shaped patch of ground colour. There are usually whitish or yellowish white dots on the lateral black pattern in III and IV stages. In V stage the entire pronotum may become black with numerous yellowish dots.

At 33° C. the mid-dorsal line on the pronotum is white, with a narrow black stripe on its either side which widens up posteriorly forming the usual black triangular metazonal patches. The lateral black pattern is less extensive, usually lighter in the prozonal region and leaving a broader comma-shaped patch of basic colour along the dorsal and posterior margin of pronotum.

At 40° C. there are narrow smoky stripes on each side of a whitish median line with the smoky triangular metazonal patches at their posterior end. The lateral black pattern is almost absent in the prozonal region and only faint smoky marks may be present in the metazonal region. At 44° C. no black pigment is left.

Meso- and meta-nota.—In II and III stages the meso and meta-nota carry a mid-dorsal black stripe in continuation of the black stripe on pronotum. Below this on each side is a whitish stripe in continuation of the similar stripe on pronotum. The remaining area below this white stripe is black.

At 24° C. and 33° C. the mid-dorsal black stripes are wider and more intense leaving a relatively narrow lateral white stripe. At 40° C. the stripes are very narrow and smoky and at 44° C. the colour is dull white.

In IV and V stages the meso and meta-notum are covered under wing pads and do not show the pattern found in the II and III stages.

Lateral thoracic sclerites.—The pro-episternum is black at 24° C. and dull white to yellowish at higher temperatures.

At 24° C. the meso-episternum and meso-epimeron have the basic colour of the body with smoky or black patches at different places.

The met-episternum and met-epimeron are uniformly black at 24° C. At 33° C. the met-episternum is mixed black and white, while met-epimeron remains uniformly black. At 40° C. the met-episternum and met-epimeron are uniformly white or yellow, with usually black marks along the sutures.

Sternum.—At 24° C. the ventral side of thorax has numerous dark brown to black patches ; at 33° C. there are smoky dots on the basic coloration, while at 40° C. the ventral thorax is almost uniformly white or yellow.

(e) *Legs*

I and II pairs.—At 24° C. and 33° C. the first two pairs of legs have numerous smoky dots with extensive black rings along the head and apex of femur and tibia. The spines are black and the tarsi, dark brownish.

At 40° C. the dots are faint brownish and only smoky spots are present on the head and apex of femur and tibia. The tarsi are lighter at 44° C.

Hind legs.—At 24° C. the anterior muscle-impression area of femur is completely black, and pigmentation is extensive. The black patterns on the anterior and posterior surfaces are almost fused dorsally with the exception of a narrow oval gap near the distal end.

At 33° C. the black pattern on the anterior muscle-impression area is less broad and leaves a narrow streak of basic colour above the antero-ventral femoral carina. It, however, possesses two black dorsal extensions near the distal end which enclose a gap of basic colour and usually end on the postero-ventral femoral carina.

At 40° C. the black pattern is narrow and covers only upper 2/3 of the anterior muscle-impression area, the remaining 1/3 along the antero-ventral femoral carina is yellowish. The two dorsal extensions at the distal end are narrow and broken and enclose a relatively larger oval gap. They end at the postero-dorsal femoral carina.

In the hoppers of the II stage at 24° C. and 33° C. the posterior muscle-impression area is also black, like the anterior one.

Markings on the meta-femur, meta-tibia and meta-tarsi undergo changes almost like those in the I and II pairs of legs. The distance between the posterior end of the femoral black pattern and the black ring at the apex of femur increases as the temperature rises.

At 44° C. no black pigment is left.

(*f*) *Abdomen*

Segment I.—In II and III stage hoppers there being no wing pads, segment I is not covered. The mid-dorsal stripe is always present with the ivory coloured triangle at the posterior margin, but the black stripe is broader and intenser at 24° C. and narrower and divided into two by a fine white median line at 33° C. and 40° C. Laterally there are a number of black dots along the posterior margin and above the ear, but their number and intensity of colour decreases as the temperature rises from 24° C. to 40° C.

In IV stage hoppers, segment I is partly covered under the wing pad and shows partial black markings. At 24° C. the mid-dorsal tergal stripe is broad and black and with the usual mid-dorsal ivory patch at the posterior margin of each segment. Laterally there are a few black dots along the posterior margin with a few more in front of it. At 33° C. the mid-dorsal stripe is faint and laterally there are only four or five dots along the posterior margin, while at 40° C. and 44° C. the black markings are absent. A few faint smoky dots may be present in the lateral aspect.

In the hoppers of V stage this segment is completely covered under the wing pads so that there are hardly any markings on it.

Segments II-VIII.—At 24° C. the dorsal black stripe is broad and undivided, with the usual white triangular area at the posterior end. Laterally above the white spiracular stripe, there is a broad straight black stripe with its anterior end much wider. Above this, is a black oblique streak and a number of black dots arranged in a row parallel to the oblique streak. Lastly there are two or three dots above this which are sometimes fused into black spots. Very little of the ground colour of the body is visible laterally. The same pattern is maintained at 33° C. and 40° C. but the streaks as well as dots become lighter and narrower.

Segments IX-X.—At 24° C. the 9th and 10th segments are almost entirely black. At higher temperatures, the mid-dorsal stripe is present in continuation of previous segments but it is narrower. The lateral aspect does not have the usual pattern consisting of regular streaks, but possesses a row or two of dots only.

Telson.—At 24° C. the telson is brown to black ; at higher temperatures it has the basic colour of the body with or without smoky spots.

Ventral surface of abdomen.—At 24° C. the ventral surface of abdomen is uniformly brown to black, excepting a lighter mid-ventral line running along the length of abdomen. At 33° C. the ventral surface is dull white with many smoky or brownish dots. At 40° C. the entire ventral surface is uniformly white or yellow.

NOTE.—The coloration of hoppers at 24° C. is the nearest approach to that of the hoppers met with normally in a swarm of spring brood, while the coloration of hoppers at 33° C. represents very nearly that of the hoppers of the summer brood.

V. Discussion

Several theories have been advanced to explain different types of colour forms among insects. Standfuss [1896] and Hudson [1913] regarded the development of pigmentation as a phenomenon of adaptation, the dark coloured forms in humid places, and near industrial towns, appearing in response to their more or less dark-coloured surroundings, providing protective coloration for the organism. The development of pigmentation in the '*gregarious*' hoppers cannot be explained on the basis of protective or concealing coloration. The '*solitary*' hoppers with their general light green colour are very well-concealed among green bushes, while the '*gregarious*' hoppers with their black and yellow colour are most conspicuous on the desert sand on which they venture out, 'without cover', in thick swarms.

Knight [1924] at first regarded the white and pigmented forms of *Perillus bioculatus* as two independent forms, but after a large number of breeding experiments he was not able to establish pure lines of non-pigmented and pigmented forms. He was, therefore, forced to the conclusion that the colour patterns of individual bugs were influenced by external conditions and were not characters inherited from their parents. This is exactly the situation with respect to the Desert Locust. External conditions determine the coloration of the hoppers, irrespective of the coloration of the parents and irrespective of the coloration, which an hopper possesses in the first stage. The most important of these external factors is, of course, the density of population in which the hoppers are bred, whether under natural conditions or in a cage. This correlation of the intensity of population and coloration greatly complicates the question of pigmentation in locusts.

Temperature also influences coloration. The production of dark colour aberrations in *Leptinotarsa* sp. at both high and very low temperatures has been explained by the failure of the action of ferments which are adversely affected at both these limits [Przibram, 1919].

Linden [1904] explains the colour variation 'of temperature forms' on the basis of changes in metabolism and particularly in the rate of respiration. According to Prochnow [1929] one of the functions of pigment-formation is the disposal of waste products. The waste products, which cannot be excreted from the

body, are deposited as pigment. From this it follows that greater the rate of metabolism, greater the quantity of waste products and higher the deposition of pigment. Schulze [1917] maintains that since the males show a more active oxidation than the females, we must have more strongly black-pigmented forms among the males than among the females. On the basis of these theories one ought to get black pigments intensifying at high temperature, but this does not happen in *Schistocerca gregaria*.

Faure [1932] investigating the causes underlying the developments of the *gregaria* black coloration in locusts proposes the theory of *locustine* production. As a result of the excessively high rate of metabolism obtaining in swarm life *locustine* is produced, and this leads to the development of black pigmentation. According to this theory increased metabolism as a result of great muscular activity at high temperature must produce larger amount of *locustine* and consequently more darkly pigmented forms, which is not the case in *Schistocerca gregaria*.

Contrary to these views, Knight [1924], basing his opinion on his experiments on *Perillus bioculatus*, maintains that increased metabolism produces de-pigmented forms. He argues that since the females have to mature large number of eggs, the metabolism in this sex goes on at a higher rate, than in the male, consequently the females are whitish between 78-82° F., while males are coloured. The physiological activity of these bugs can also be raised by rearing them at a temperature of 85° F. or higher, when the white forms appear. He maintains that restricted movements, in other words, less exercise, also gives darker forms. Therefore according to this investigator increased metabolism does not produce pigment, but on the contrary, consumes or otherwise disposes of the materials, which under low temperature are laid down as pigment in the body wall. This view is also supported by the studies of Genieys [1925], Schlottke [1926], and Kühn and Henke [1929].

According to Schroder [1903], dark pigments are formed under the influence of cold so as to increase the absorption of heat by the insect and thus counterbalance the influence of cold on development. This theory does not explain the origin of pigmentation ; it only explains the consequence of the development of pigmentation.

The influence of temperature on the pigmentation of Desert Locust hoppers bred crowded seems to be exactly like that mentioned by Knight. More deeply pigmented forms are produced at low temperature, while less pigmented forms result from breeding at high temperature. In regard to the influence of muscular activity, however, the results of experiments conducted [Afzal Husain and Mathur, 1936] are very different. Increased muscular activity in their experiments produced darker forms.

These studies thus show that whatever the significance of the development of black pigmentation, the causes leading to it are physiological and not morphological. And since the effect of temperature on physiological processes is different

in different groups of insects it would not be safe to make generalization, each group of insects meriting special study.

In the Desert Locust, as in some other locusts, the *swarming* phase differs from the *solitary* phase, in addition to coloration, in some anatomical details, expressed in differences in the shape and size of certain portions of the body. It will be worth investigating if coloration and anatomical differences go together. This problem will receive attention in a subsequent paper. It appears evident that one cannot place too much reliance on coloration in distinguishing phases and sub-phases.

VI. Summary

The temperature at which locust hoppers are bred has a marked influence on the intensity as well as the extent of pigmentation in the hoppers of *Schistocerca gregaria* bred crowded.

Hoppers bred crowded at 24° C. develop deep and extensive black patterns on the various parts of the body and the black colour is predominent.

At high temperature the black markings get reduced and the basic yellow or greenish yellow colour predominates.

At a still higher temperature of 44° C. the black markings are almost entirely absent, and the hoppers are whitish.

The present studies indicate that the causes leading to pigmentation are physiological and not morphological. The influence of temperature on pigmentation would therefore be determined by the manner in which the physiological processes are affected in different groups of insects.

These experiments also show that all stages in the variation in colour from black to white can be produced in the crowded hoppers under the influence of temperature. Incidently one may ask if reliance can be placed on colour in making distinction between the different phases and sub-phases.

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TABLE I

Life-history of hoppers reared at different constant temperatures . Experiment I
Reference No. L 224

Date	Days	24° C.	24° C.	27° C.	30° C.	30° C.	33° C.	33° C.	36° C.	36° C.	36° C.	40° C.
11th Nov. 1932 .	0	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)
12th „ . .	1											
13th „ . .	2											
14th „ . .	3									II (3)		II (3)
15th „ . .	4						II (3)	II (3)	II (3)		II (3)	
16th „ . .	5				II (3)	II (3)						D (1)
17th „ . .	6			II (3)								III (2)
18th „ . .	7				D (1)				III (3)	III (3)	III (3)	
19th „ . .	8	II (3)					III (3)	III (3)				
20th „ . .	9		II (3)									
21st „ . .	10		D (1)		III (2)	III (3)						IV (2)
22nd „ . .	11			D (1)					IV (3)	IV(3)	IV (3)	
23rd „ . .	12			III (2)			IV (3)	IV (3)				
24th „ . .	13											
25th „ . .	14					D (1)					D (1)	V (2)
26th „ . .	15				IV (2)	IV (2)			V (3)	V (3)	V (2)	

TABLE I—*contd.*

Date	Days	24° C.	24° C.	27° C.	30° C.	30° C.	33° C.	33° C.	36° C.	36° C.	36° C.	40° C.
27th Nov. 1932 .	16	III (3)	III (2)							D (1)		
28th „ . .	17						V (3)	V (3)				
29th „ . .	18											
30th „ . .	19			IV (2)								
1st Dec. 1932 .	20				V (2)	V (2)						
2nd „ . .	21											A (2)
3rd „ . .	22								A (3)	A (2)	A (2)	
4th „ . .	23											
5th „ . .	24							D (1)				
6th „ . .	25											
7th „ . .	26	IV (3)					A (3)	A (2)				
8th „ . .	27		IV (2)	D (1)								
9th „ . .	28			V (1)								
10th „ . .	29											
11th „ . .	30											
12th „ . .	31				A (2)	A (2)						
13th „ . .	32											
14th „ . .	33											

15th „ . .	34													
16th „ .	35													
17th „ . .	36													
18th „ . .	37													
19th „ . .	38													
20th „ . .	39	V (3)												
21st „ . .	40		V (2)											
22nd „ . .	41													
23rd „ . .	42													
24th „ . .	43													
25th „ . .	44													
26th „ . .	45													
27th „ . .	46													
28th „ . .	47			A (1)										
29th „ . .	48													
30th „ . .	49													
31st „ . .	50													
1st Jan. 1933 .	51													
2nd „ . .	52													
3rd „ . .	53	D (1)												
4th „ . .	54													
5th „ . .	55													
6th „ . .	56													

TABLE I—*concld.*

Date	Days	24° C.	24° C.	27° C.	30° C.	30° C.	33° C.	33° C.	36° C.	36° C.	36° C.	40° C.
7th Jan. 1933 .	57											
8th " . .	58											
9th " . .	59	D (1)										
10th " . .	60											
11th " . .	61											
12th " . .	62	A (1)	A (2)									
Duration of hopper stage in days		62	62	47	31	31	26	26	22	22	22	21

Roman numbers represent instars of the hoppers. Arabic numbers in ( ) represent the number of hoppers in the cage.
D=Died and figure in ( ) represents number dead. A=Adult, and figure in ( ) represents their number.

TABLE II

Life-history of hoppers reared at different constant temperatures. Experiment II
Reference No. P 74

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
14th Jan. 1933 .	0	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)
15th „ . . .	1						
16th „ . . .	2	D (1)					
17th „ . . .	3			D (1)		II (3)	II (3)
18th „ . . .	4			II (2)	II (3)		
19th „ . . .	5						
20th „ . . .	6		II (3)			III (3)	III (3)
21st „ . . .	7						
22nd „ . . .	8	II (2)			III (3)		
23rd „ . . .	9			III (2)			D (1)
24th „ . . .	10					IV (3)	IV (2)
25th „ . . .	11						
26th „ . . .	12		III (3)		IV (3)		
27th „ . . .	13						
28th „ . . .	14			IV (2)		V (3)	V (2)
29th „ . . .	15						
30th „ . . .	16	III (2)					
31st „ . . .	17				V (3)		
1st Feb. 1933 . .	18		D (1)				
2nd „ . . .	19		IV (2)				
3rd „ . . .	20			V (2)			
4th „ . . .	21					A (3)	A (2)
5th „ . . .	22						

TABLE II—*contd.*

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
6th Feb. 1933 .	23						
7th ,, . . .	24						
8th ,, . . .	25						
9th ,, . . .	26				A (3)		
10th ,, . . .	27	IV (2)	V (2)				
11th ,, . . .	28						
12th ,, . . .	29						
13th ,, . . .	30						
14th ,, . . .	31			A (2)			
15th ,, . . .	32						
16th ,, . . .	33						
17th ,, . . .	34						
18th ,, . . .	35						
19th ,, . . .	36						
20th ,, . . .	37						
21st ,, . . .	38						
22nd ,, . . .	39	D (1)					
23rd ,, . . .	40	V (1)					
24th , . . .	41						
25th ,, . . .	42						
26th ,, . . .	43						
27th ,, . . .	44						
28th ,, . .	45						
1st Mar. 1933. .	46		A (2)				
2nd ,, . . .	47						
3rd ,, . . .	48						

TABLE II—*concld.*

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C
4th Mar. 1933 .	49						
5th „	50						
6th „	51						
7th „	52						
8th „	53						
9th „	54						
10th „	55						
11th „	56						
12th „	57						
13th „	58						
14th „	59						
15th „	60						
16th „	61						
17th „	62						
18th „	63	A (1)					
Duration of hopper stage (days).		63	46	31	26	21	21

TABLE III.

Life history of hoppers reared at different constant temperatures Experiment III
Reference No. L 276

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
16th Jan. 1933 .	0	I (7)	I (7)	I (7)	I (7)	I (7)	I (7)
17th „ . . .	1	D (1)	D (2)				D (1)
18th „ . . .	2						
19th „ . . .	3					II (7)	II (6)

TABLE III—*contd.*

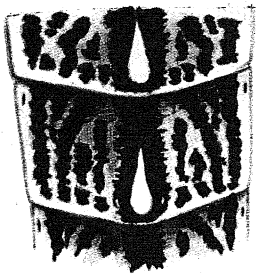
Date	Days	24° C.	27° C.	30° C.	33° C	36° C.	40° C
20th Jan. 1933 .	4				II (7)		
21st ,, . . .	5			II (7)			
22nd ,, . . .	6		II (5)			III (7)	III (6)
23rd ,, . . .	7						
24th ,, . . .	8	II (6)			D (2)		
25th ,, . . .	9	D (2)		III (7)	III (5)		
26th ,, . . .	10					IV (7)	IV (6)
27th ,, . . .	11						
28th ,, . . .	12		D (1)			D (1)	
29th ,, . . .	13		III (4)		IV (5)	D (1)	D (1)
30th ,, . . .	14			IV (7)		V (5)	V (5)
31st ,, . . .	15						
1st Feb. 1933 . .	16						
2nd ,, . . .	17	III (4)					
3rd ,, . . .	18				V (5)		
4th ,, . . .	19						D (1)
5th ,, . . .	20		IV (4)	V (7)			
6th ,, . . .	21					A (5)	A (4)
7th ,, . . .	22						
8th ,, . . .	23						
9th ,, . . .	24			D (1)			
10th ,, . . .	25						
11th ,, . . .	26						
12th ,, . . .	27	D (1)			A (5)		
13th ,, . . .	28	IV (3)					
14th ,, . . .	29		V (4)				

TABLE III—*contd.*

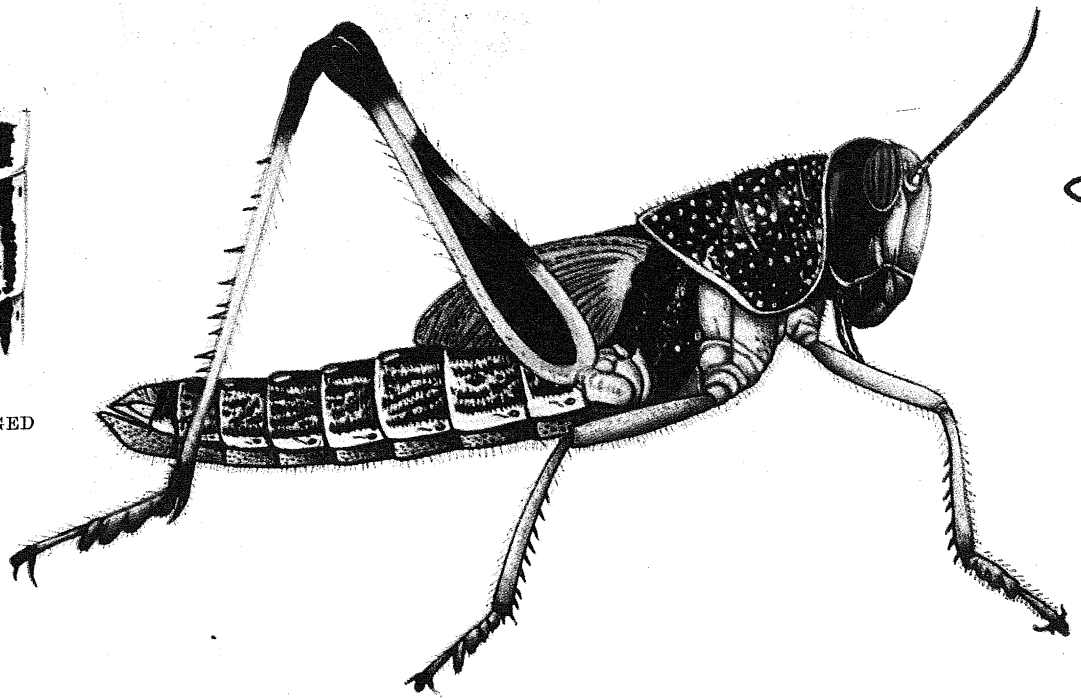
Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
15th Feb. 1933 . .	30						
16th „ . . .	31			A (6)			
17th „ . . .	32						
18th „ . . .	33						
19th „ . . .	34						
20th „ . . .	35						
21st „ . . .	36						
22nd „ . . .	37						
23rd „ . . .	38		D (1)				
24th „ . . .	39						
25th „ . . .	40						
26th „ . . .	41	V (2)					
27th „ . . .	42						
28th „ . . .	43						
1st Mar. 1933 . .	44						
2nd „ . . .	45						
3rd „ . . .	46						
4th „ . . .	47		A (3)				
5th „ . . .	48						
6th „ . . .	49						
7th „ . . .	50						
8th „ . . .	51						
9th „ . . .	52						
10th „ . . .	53						
11th „ . . .	54						
12th „ . . .	55						

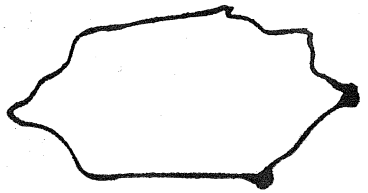
TABLE III—*concld.*

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
13th Mar. 1933 . .	56						
14th ,, . . .	57						
15th ,, . . .	58						
16th ,, . . .	59						
17th ,, . . .	60						
18th ,, . . .	61						
19th ,, . . .	62						
20th ,, . . .	63						
21st ,, . . .	64	A (2)					
Duration of hopper stage in days.		64	47	31	27	21	21



ABDOMINAL SEGMENTS ENLARGED





CROSS SECTION AT ab.

1. Frons.
2. Clypeus.
3. Labrum.
4. Vertex.
5. Gena.
6. Subocular genal stripe.
7. Post gena.
8. Compound eye.
9. Antenna.
10. Mandible.
11. Maxillary palp.
12. Labial palp.
13. Post-ocular patch.
16. Keel
17. Metazonal triangular patch.
18. Lateral black patch.
19. Wing pad.
20. Meso-episternum.
21. Meso-epimeron.
22. Met-episternum.
23. Met-epimeron.
24. Leg, I pair.
25. Leg, II pair.
26. Leg, III pair.
27. Hind femur.
28. Anterior muscle impression.
31. Tibial spines.
32. Claws.
33. Tarsi.
34. Abdomen.
35. Mid-dorsal black stripe.
36. White mid-dorsal triangular area.
37. Lateral spiracular stripe.
38. Telson and Cerci.
39. Spiracle.
40. Lateral straight stripe.
41. Lateral oblique streak.
42. Antro-dorsal carina.
43. Antro-ventral carina.
44. Postero-dorsal carina.

GREGARIOUS HOPPERS BRED CROWDED AT A TEMPERATURE OF 24°C.

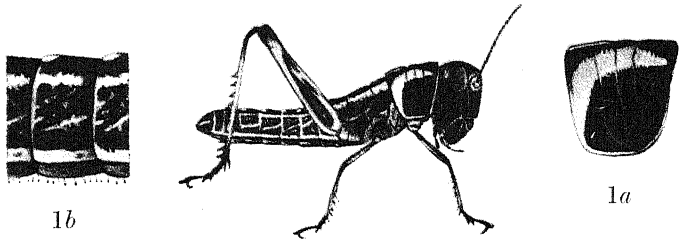


FIG. 1. Hopper of the second stage (×2·2).

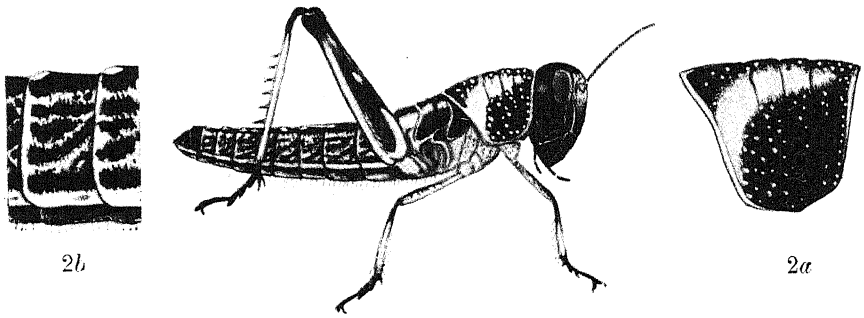


FIG. 2. Hopper of the third stage (×1·8).

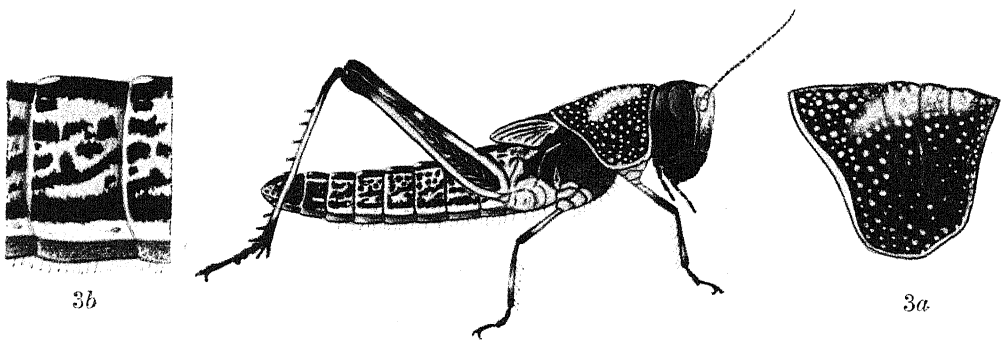


FIG. 3. Hopper of the fourth stage (×1·5).

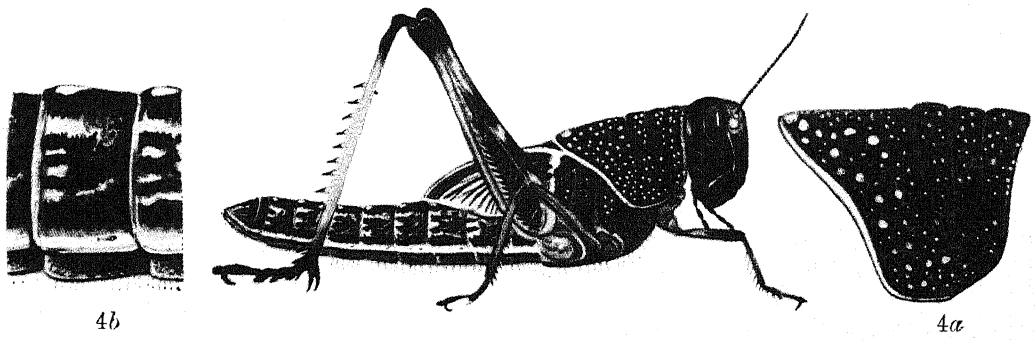
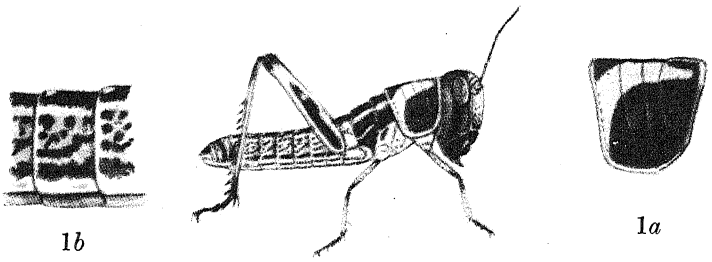


FIG. 4. Hopper of the fifth stage (×1·1).

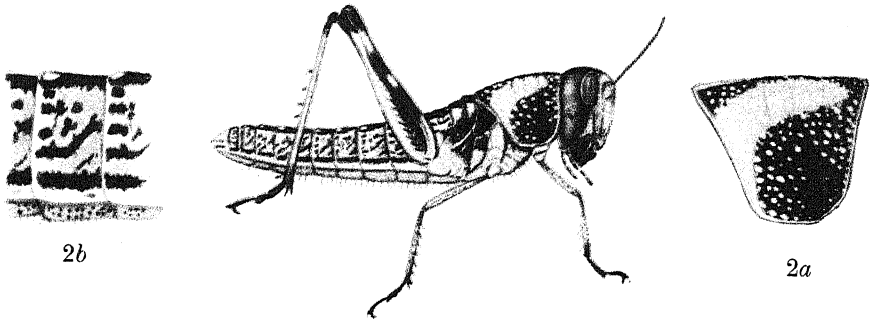
a. Pronotum enlarged, *b*. Abdominal segments enlarged.

GREGARIOUS HOPPERS BRED CROWDED AT A TEMPERATURE OF 33°C.



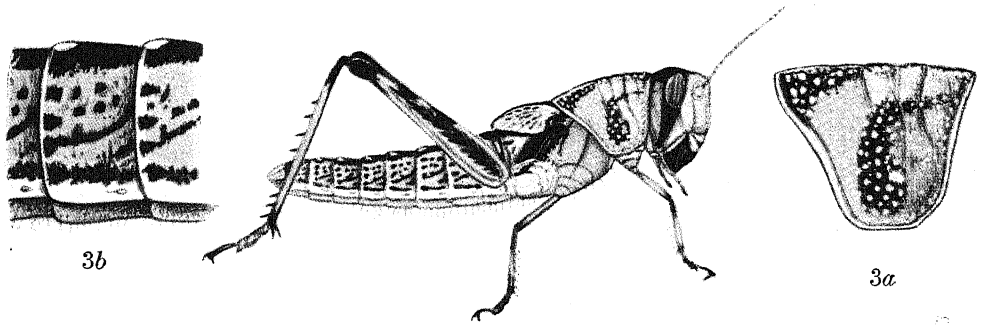
1*b* 1*a*

FIG. 1. Hopper of the second stage (×2·2).



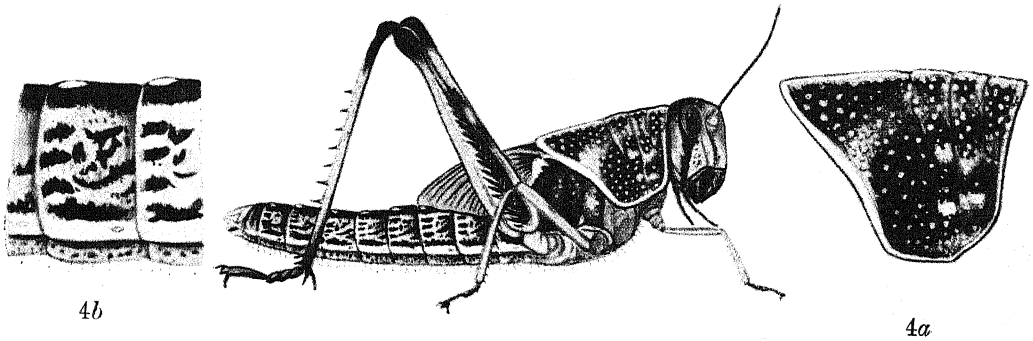
2*b* 2*a*

FIG. 2. Hopper of the third stage (×1·8).



3*b* 3*a*

FIG. 3. Hopper of the fourth stage (×1·5).



4*b* 4*a*

FIG. 4. Hopper of the fifth stage (×1·1).

a. Pronotum enlarged, *b*. Abdominal segments enlarged.

6

GREGARIOUS HOPPERS BRED CROWDED AT A TEMPERATURE OF 40°C.

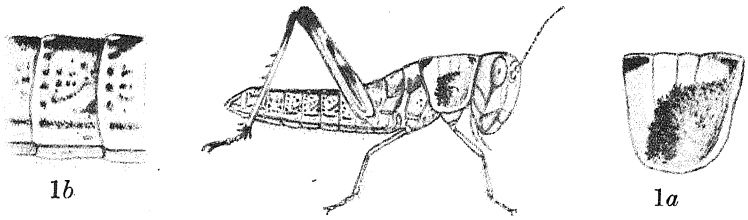


FIG. 1. Hopper of the second stage (×2·2).

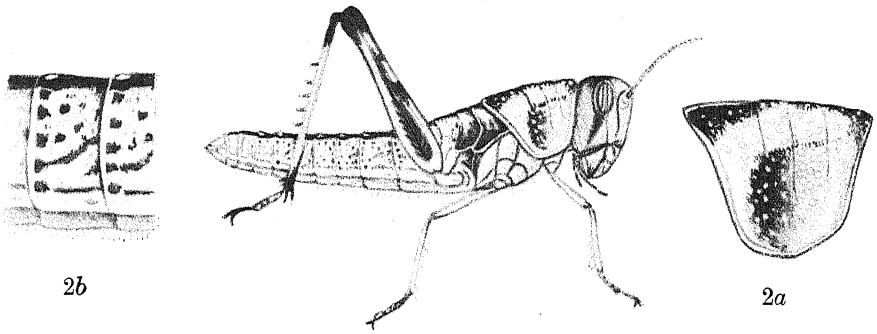


FIG. 2. Hopper of the third stage (×1·8).

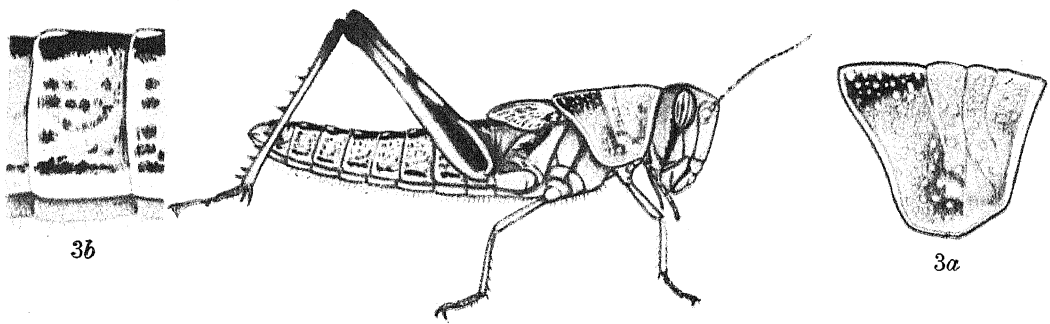


FIG. 3. Hopper of the fourth stage (×1·5).

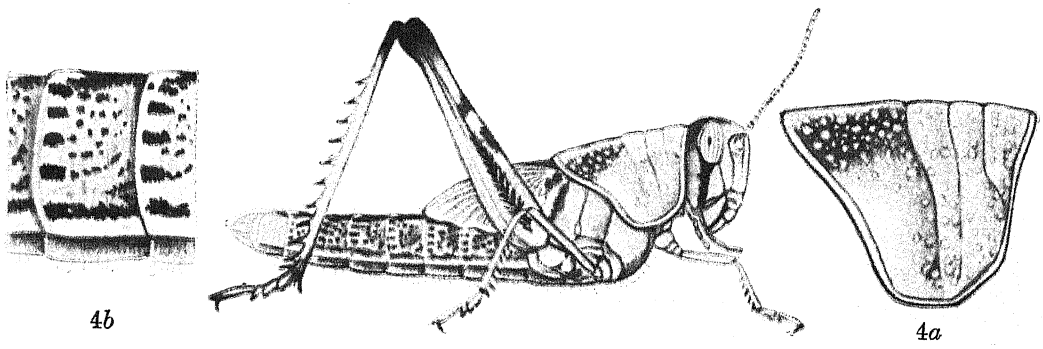
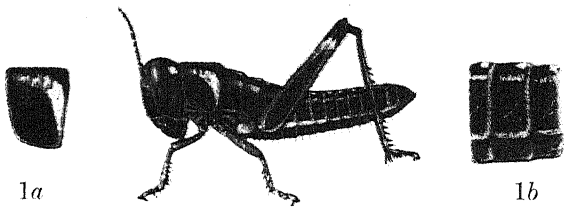


FIG. 4. Hopper of the fifth stage (×1·1).

(*a*) Pronotum enlarged, (*b*) Abdominal segments enlarged.



1*a* 1*b*

FIG. 1. Hopper of the first stage, which was the starting point of experiment (×2·8)

(*a*) Pronotum enlarged (*b*) Abdominal segments enlarged.

HOPPERS BRED CROWDED AT 44° C.

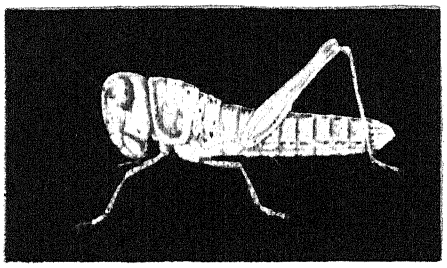


FIG. 2. Hopper of the second stage (×2·8)

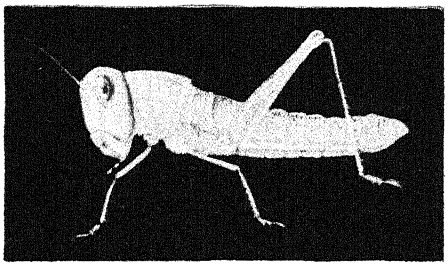


FIG. 3. Hopper of the third stage (×1·7)

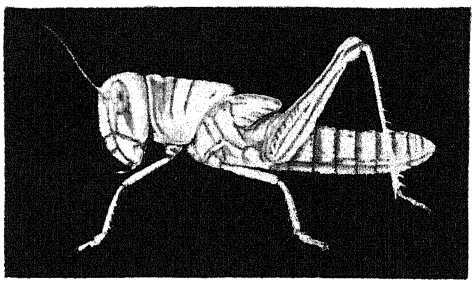


FIG. 4. Hopper of the fourth stage (×1·7)

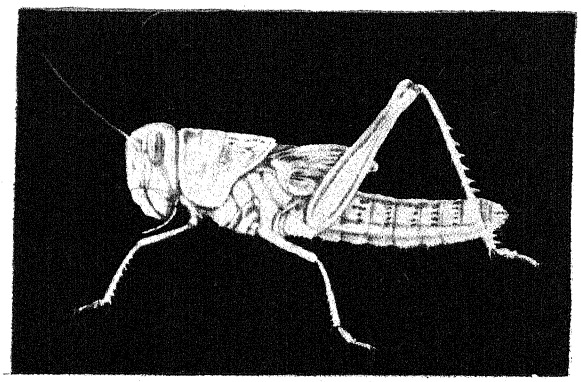


FIG. 5. Hopper of the fifth stage (×1·2)

STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

*VII. FACTORS DETERMINING THE MOVEMENT OF THE VERMIFORM LARVAE

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AND

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(Received for publication on 6th January 1936)

(With four text-figures)

INTRODUCTORY

Like most other Locustidae (Acridiidae), *Schistocerca gregaria* Forsk. normally lays its eggs in holes made in the soil. These burrows ordinarily are about 8-13 cm. deep. The eggs are deposited in an elongated pod at the blind end of this burrow. While the eggs are being passed out of the body of the female, a viscous matter is also poured out, and with the air bubbles present, it forms a foamy material which sets as a frothy or spongy covering of the egg-pod. After all the eggs have been laid the female gradually withdraws its abdomen from the burrow and while doing so she pours out a mass of this foamy material which sets into a frothy or spongy plug or stopper, which occupies the entire space in the burrow not occupied by the egg-pod. The eggs thus lie a few centimeters below the soil surface, in a

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*Studies on *Schistocerca gregaria* Forsk.

I. The Micropyle in *Schistocerca gregaria* Forsk. and some other Acridiidae. *Ind. J. Agric. Sci.* **3**: 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6**: 188-262.

III. Why do Locusts Eat Wool? A Study in the Hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6**: 263-67.

IV. Colour Changes and Sexual Behaviour in Desexualised *Schistocerca gregaria* Adults. *Ind. J. Agric. Sci.* **6**: 586-90.

V. Pigmentation and Physical Exertion. *Ind. J. Agric. Sci.* **6**: 591-623.

VI. Influence of Temperature on the Intensity and Extent of Black Pattern in the Desert Locust Hoppers bred Crowded. *Ind. J. Agric. Sci.* **6**: 624-664.

burrow closed with a spongy plug. This spongy plug may sometimes come slightly above the surface of the soil, but generally it stops a little below the upper opening of the burrow. Very often the mouth of the burrow gets closed by the disturbance of the soil.

From what has been said above, it is evident that the larvae hatching a few centimeters below the surface of the soil have to work their way out.

As is well known, the insect as it comes out of the egg shell or chorion is not a hopper but a vermiform larva. In other words the future hopper is enclosed in a thin membraneous overall, and its appendages lie in small blind sleeves of this overall, and are not capable of free movement. The only method of progression, which this vermiform larva is capable of, is wriggling like a worm ; hence the name vermiform larva. These larvae thus wriggle their way out through the frothy plug and the soil above and reach the surface where the 'intermediate' moult occurs and the larval skin is shed and the first instar hoppers emerge.

The question arises : What directs the vermiform larvae towards the surface of the soil ? The present state of our knowledge regarding this interesting phenomenon has been admirably summarised by Uvarov [1928] in the following :—

" The causes that direct the movement of the emerging larvae upwards have never been studied, although the phenomenon is of considerable interest. Theoretically it may be suggested that phototropism of the larvae plays a certain role, though on the other hand, larvae hatching in a glass jar containing earth move upwards along its transparent walls as usual. Apparently the movement is influenced by gravity (negative geotropism) and this suggestion is supported by the fact that the larvae that emerge from an egg turned upside down also move upwards ."

Thus two forces have been credited as providing the stimulus—positive phototropism and negative geotropism. Our experiments, however, show that an absolutely different factor is responsible, for directing the movement of the larvae out of the soil.

Positive phototropism

The fact, that the vermiform larvae in the soil, particularly when the opening of the burrow is closed, cannot be directed by light, is in itself a definite proof that phototropism plays no role in this directive movement. During our investigation numerous cages were kept in incubators placed in a photographic dark room, and it was found that the emergence of the vermiform larvae from the soil was in every case quite normal.

Negative geotropism

Definite experiments have been performed to determine if geotropism is the directive factor, but the results obtained show that it is not so.

Experiment 1.—A fertilized female was caged in a circular glass dish 15 cm. in diameter and 9·5 cm. deep (Fig. 1). At the bottom of the dish a layer of moist soil 3·9 cm. in thickness was placed. On 23rd February 1931 the female had bored into the soil almost vertically and having come against the bottom of the dish she continued to bore parallel to the bottom. She thus made a vertical burrow 3·9 cm. deep and a horizontal burrow 8·6 cm. long, along the bottom of the dish. Eggs were laid at the blind end of this burrow. The egg-pod which was 5·1 cm. long was parallel to the bottom of the dish and consequently the long axis of the eggs was horizontal.

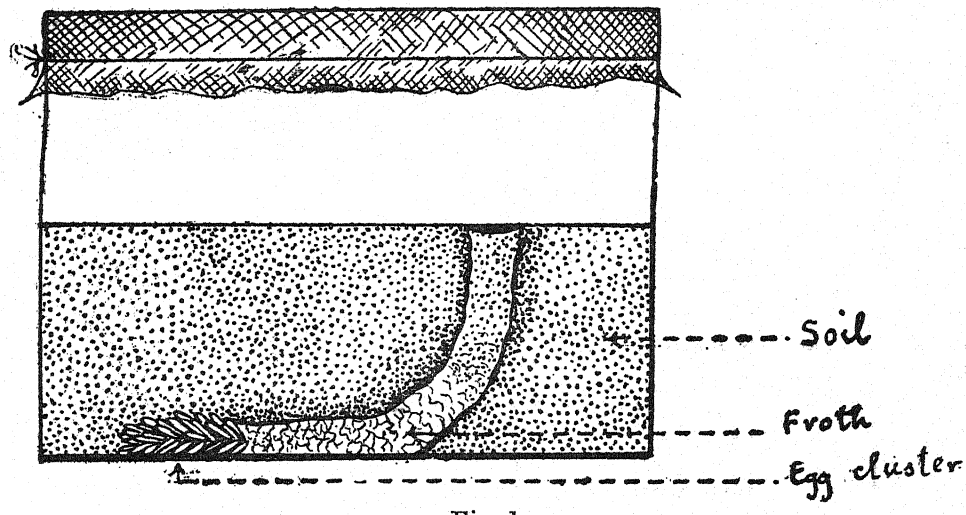

Fig. 1.

The remainder of the horizontal limb of the burrow was closed by the plug of froth 3·5 cm. in length. This plug reached the end of the horizontal portion of the burrow. The vertical part of the burrow got closed on account of the soili n the dish being moistened with water from time to time.

At 12-10 hours on 20th March 1931 when the dish was examined the eggs were hatching. The movements of the larvae during their attempt to come out of the burrow were studied. At the time of observation six larvae were seen in a line near the junction of vertical and the horizontal limbs of the burrow, trying to force their way out. Failing in their attempt to make a path through the soil, at about 1-45 P. M. the larvae turned back towards the blind end of the burrow where the egg-pod lay. By this time more of the larvae had hatched out and had reached the froth. Thus the larvae in front being unable to go back, moved in a circle at the end of the horizontal limb of the burrow. After about two and a half hours the dish was placed on edge in such a manner that the egg-pod was in a vertical position with the plug of forth, downwards. The larvae did not make any attempt to go up towards the egg-pod. They did not show any response to go against the

force of gravity but continued to move in a circle and those behind continued to move down in the plug of froth. In other words the movements were quite independent of the directive force of gravity.

As the soil was rather hard for the larvae to make their way through, a hole was made for them to come out. The vermiform larvae now came out of this hole and shed their skin.

Experiment 2.—A fertilized female was made to lay eggs in an ordinary glass test-tube filled with soil. The egg-pod was visible through the walls of the tube. The length of the egg-pod was 2·5 cm. The plug of froth which extended to the surface of the soil was 6 cm. of which the lower 4 cm. could be seen clearly. The closed bottom end of this tube was broken. The length of the column of sand between this end and the bottom of the egg-pod was 6·4 cm. This tube containing the egg-pod was placed upside down, over a glass chimney so that the egg-pod was still held in a vertical position but its normal lower end was above, and the plug of froth below (Fig. 2). Except this the egg-pod was not disturbed in any way.

The eggs hatched on 14th April 1931 and all the fifty-two larvae worked their way downwards through the plug of froth and dropped down at the bottom of the glass chimney, the intermediate moult taking place in this situation.

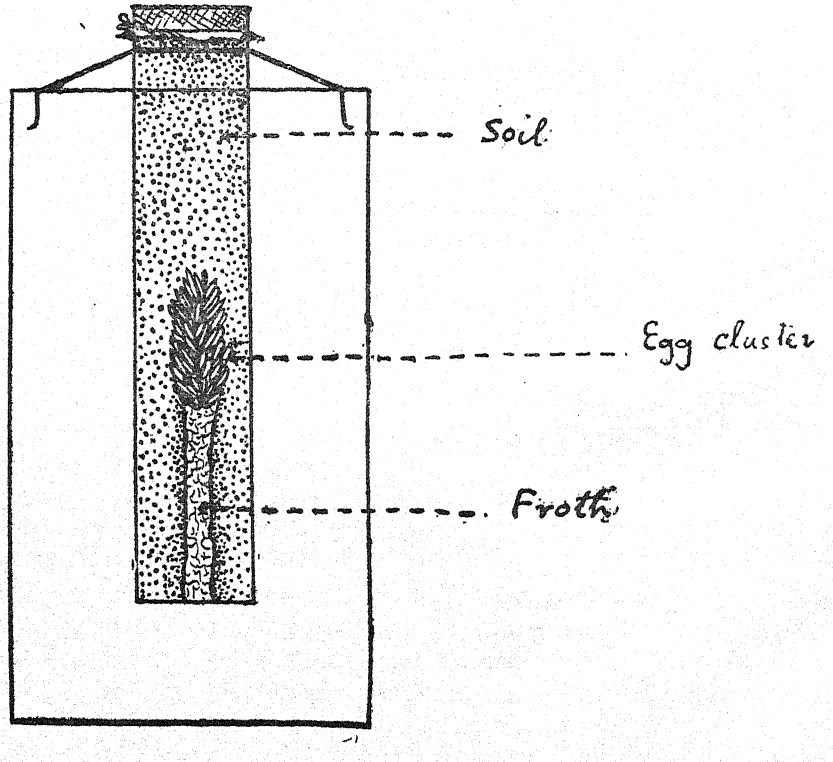

Fig. 2.

The larvae did not work their way upwards as should have happened if negative geotropism had been the determining factor; they all bored their way downwards through the froth.

Experiment 3.—An egg-pod deposited on 7th April 1931 was removed from the soil on 15th April 1931 and placed in a tube made of celluloid, 14 cm. long and 2·4 cm. in diameter (Fig. 3). The egg-pod was 3 cm. long. The tube was placed over a glass chimney (as in experiment 2) in such a manner that the normal upper end of the egg-pod was uppermost and was covered with 4 cm. of sand. At the lower end of the pod a cork (cork No. 1) with a hole in the centre was placed to keep the egg-pod in position. Through this hole in the cork, the original froth column was introduced so as to come in contact with the eggs at the normal lower end of the egg-pod. Round this frothy plug, soil was put and another cork (cork No. 2) with a hole introduced at the lower end of the tube to keep this soil in position.

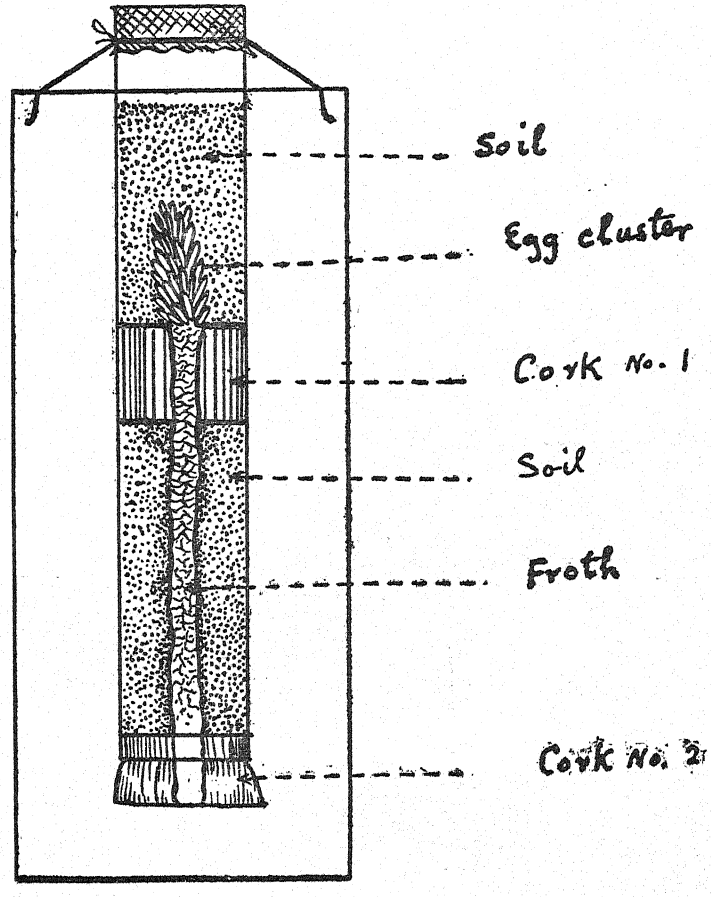

Fig. 3.

The eggs hatched on 19th April 1931 and all the larvae worked their way downwards and passed through the hole in the upper of the two corks. Most of them went round and round in a circle in the space just below this cork. Two of them had almost reached the lower cork, none of them succeeding in finding an opening through the lower cork. None of the larvae went to the upper end of the egg-pod.

This experiment shows that if the froth column is removed from the normal upper end of the egg-pod and placed at the normal lower end of the pod, then the vermiform larvae bore through it and work downwards instead of going upwards.

Experiment 4.—A four-way celluloid tube was prepared. The diameter of the tube was one inch and each limb was $2\frac{1}{2}$ in. long. The four limbs of the cross so formed were marked A, B, C and D (Fig. 4). An egg-pod (without froth) which had been laid in soil in nature (Shergarh in Montgomery District on 15th August 1931) was placed at 12 noon in the central space in the direction of the tube A-B. Two centimetre thick layer of moist soil was put in the four limbs around the egg-pod and the tube placed horizontally. Muslin was tied at the free ends of the tubes.

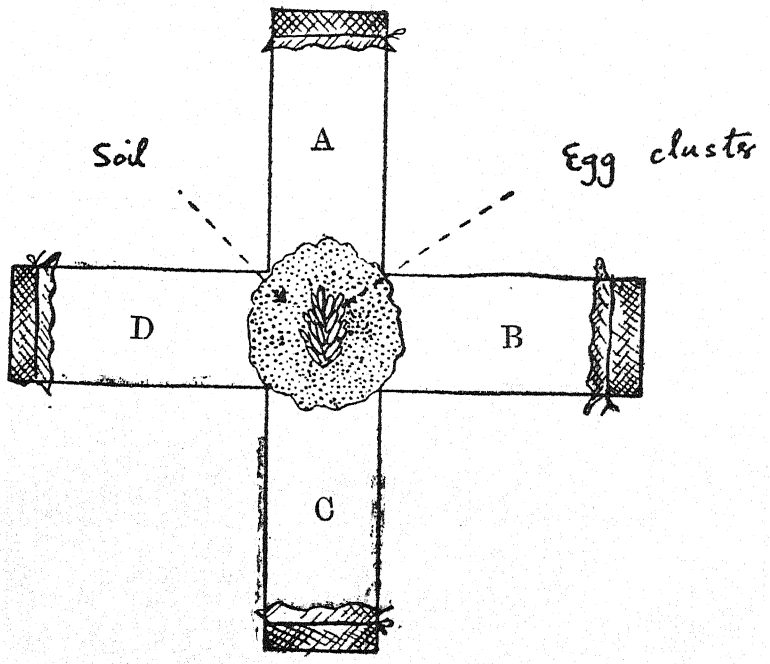

Fig. 4.

Hatching started on the same day at 13 hours and by 18 hours the hoppers had worked their way through the soil into the different limbs of the tube as follows :–

Limb A	6 hoppers.
,, B	4 hoppers.
,, C	7 hoppers.
,, D	3 hoppers.

This experiment shows that if an egg-pod is placed in the soil in such a way that the pressure of soil on all sides is the same, then the larvae after hatching may take any direction.

It has been seen that sometimes eggs are laid in vertical clay-cliffs, locally known as *khundars*, and these burrows being almost at right angles to the surface of the cliff are horizontal in position. In such cases the larvae work their way horizontally and drop down at the foot of the cliff. The larvae hatching out from such a position have actually been observed.

Conclusions

From these experiments we have arrived at the conclusion that light and gravity play no part in directing the outward movement of the emerging larvae from the soil.

The vermiform larvae after hatching take the path of least resistance and this path, in nature, is provided by the frothy plug deposited at the upper end of the egg-pod. It is through this plug that the larvae work their way up. In other words the direction of the movement of the larvae is determined by pressure ; they move in the direction the pressure is least.

Summary

The existing idea that the movement of the larvae of locusts out of soil is determined by positive phototropism or negative geotropism of the larva is not correct, the direction of their movement is determined by pressure. They move in the direction of least pressure, or in other words they follow the path of least resistance.

REFERENCE

Uvarov, B. P. (1928). Locusts and Grass-hoppers. London, pp 352.

STUDIES IN PLANT BREEDING TECHNIQUE

I. AN ANALYSIS OF THE EFFICIENCY OF SELECTION METHODS USED IN THE IMPROVEMENT OF MALVI COTTON

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Institute of Plant Industry, Indore, Central India

(Received for publication on 11th February 1936)

CONTENTS

		Page
I.	Introduction	672
II.	Material and results	673
III.	Methods	674
IV.	Discussion	678
V.	Summary	683
	Reference	683

I. Introduction

The work here discussed was commenced at the Institute of Plant Industry by Mr. (now Sir) A. Howard and Mrs. Howard in 1925. The aims they had in view are given in their account of the first five years' work of the Institute [Howard and Howard, 1929], and were for Malvi cotton as follows; "the isolation of a robust, rapidly maturing type with a fibre capable of spinning at least twenty counts which will be suitable for dry cultivation on the Malwa plateau".

At that time it was believed that the Malvi crop had suffered a serious deterioration as a result of mixing with imported *roseum* seed, and consequently some trouble was taken to obtain seed from isolated tracts where mixing was less easy as a nucleus stock for improvement. Kannod in the Nemawar district of the Holkar State was chosen as such an isolated tract. While geographically it belongs to the Nimar tract, from the point of view of crop ecology it appears to belong to Malwa, and it grows a Malvi type of cotton. Owing to the lack of communication between Nemawar and neighbouring regions of the Central Provinces it was believed that little seed exchange had taken place. It has since been found that the invasion of *roseum* into Malwa has been of negligible importance.

The unselected mixed Malvi crop varies in halo-length from 14 to 22 mm., gins on an average about twenty-nine per cent and has a spinning value of 10-12 H. S. W. C., on the Indian Central Cotton Committee's Technological Laboratory's scale. It is also somewhat late, and the third picking is often lost when frosts occur in mid-January.

II. Material and results

A foundation stock was built up by single-plant selection of Malvi type plants, (1) in 1924-25 direct from cultivators' fields in various villages up to about twenty miles from Indore, and (2) in 1925-26 from Institute fields sown with seed from Kannod.

By progeny-row breeding a series of strains was established, and these were steadily reduced in number by selection both between and within strains until in 1932-33, five remained. Yield testing was commenced in 1931-32, when a replicated, but not randomised trial was carried out with ten strains. In 1932-33, and 1933-34, the five remaining strains were tested against local in randomised block trials in various parts of Malwa, and as a result three more strains were discarded. In 1934-35 the remaining two strains were tested throughout Malwa against local and such improved varieties from neighbouring provinces as appeared likely to do well under black soil conditions, and were proved to be greatly superior both to the local control and to all possible outside competitors. In 1934-35 seed of one of the two final strains, Malvi 1 was distributed to member states in Malwa, and in 1935-36 seed of both strains, Malvi 1 and Malvi 9, was distributed. Further distribution will be of Malvi 9 only. Spinning tests were carried out by the Indian Central Cotton Committee's Technological Laboratory on these two strains with the local as control, in 1932-33, 1933-34, and 1934-35. Data for commercial characters of Malvi 1, Malvi 9 and local (control) are summarised in Table I for the years 1932-33 to 1934-35, during which time all field trials were carried out in randomised replicated layouts.

Table I

Commercial characters of Malvi 1, Malvi 9 and local

Strain	No. of trials	Halo length (mm.)	Ginning per cent	Yield (lbs. per acre)	Percentage picked before frost	Spinning tests	
						No. of tests	H.S.W.C.
1932-33—							
Local . .	4	19·8	28·7	484	91·1	3	12·2
Malvi 1 . .	4	21·7	29·3	580	93·7	3	13·3
Malvi 9 . .	4	22·5	32·3	554	91·7	3	15·5
1933-34— ..							
Local . .	6	21·3	29·6	330	85·6	2	11·5
Malvi 1 . .	6	22·9	31·0	409	91·6	5	12·2
Malvi 9 . .	6	23 2	33·3	413	89·6	4	16·8
1934-35—							
Local . .	7	23·6	28·5	325	63·3	..	..
Malvi 1 . .	7	23·9	29·3	332	74·3	2	14·0
Malvi 9 . .	7	24·3	31·9	357	65·1	2	20·0
Mean increase over local—							
Malvi 1 . .	..	1·3	0·9	60·7	6·5	..	0·9
Malvi 9 . .	..	1·8	3·6	61·7	2·1	..	4·3

Inspection of Table I shows that both strains are better than the local control in all characters, and, in all characters except earliness and possibly yield, Malvi 9 is better than Malvi 1. The data are summarised from records of seventeen trials, in most of which the differences observed were significant, so that the differences in the means can be relied on as representing real differences between the strains. The increase in value to the cultivator, obtainable by substituting Malvi 9 for local may be estimated at about thrity-five per cent with no allowance for premium due to enhanced spinning value and about fifty per cent including premium for enhanced spinning value. The fact that marked improvement has been obtained in all the major commercial characters has led to a widespread and insistent demand for seed, and the difficulties usually experienced in distributing seed of a strain dependent for its superiority on receiving a premium for high spinning value are not being encountered. In addition, prominent representatives of the Indore and Bombay mill industries have urged its rapid spread as promising an improvement of first importance in the quality of the Malvi crop. As a plant-breeding project, therefore, the work under discussion is abundantly justified by its results.

The interest of the selection records lies in the information they provide concerning the efficiency of the methods adopted. Two strains have been isolated which are markedly superior to the local. Does this achievement represent the maximum possible exploitation of the material examined, or were there genetic potentialities in it which the methods adopted were not sufficiently selective to reveal ?

III. Methods

Of the characters studied, vigour was given first importance throughout the period and the number of bolls per plant was ranked second. Of other yield characters weight of *kapas* per plant, and weight of lint per plant and lint index were studied and selections based on them in some seasons. Earliness was estimated throughout by the date of opening of the first boll, and in some years by the date of the first flower or the date of the last picking. It was considered of less importance than yield. In general, only those families which were judged in the field as satisfactory in vigour, yield and earliness were carried on for study of lint characters in the laboratory.

Of the lint characters studied, "feel" (fine or coarse) and ginning percentage were considered together, as they were found to be to a large extent mutually exclusive, and it was desired to combine as far as possible a soft lint with a high ginning percentage. These two characters were ranked as of greater importance than lint length which was judged by maximum halo-length.

In Tables II to V are summarised the selection data on four representative characters ; bolls per plant, date of bursting of first boll, ginning percentage, and halo-length, for the period 1927-28 (halo-length from 1926-27) to 1933-34.

In following the course of the strain means for yield and earliness from year to year, it is clear that other factors besides selection have influenced them largely. On bolls per plant the most important environmental influence is the incidence o late rains causing shedding, and it is clear from Table II that the major fluctuations in boll number were due to this factor and not to selection. Late rains affect the date of first boll bursting similarly, and for the same reason. Ginning percentage figures run consistently. Halo-length means gave a large jump between 1927-28 and 1928-29, and differed very little from each other in subsequent years.

The effect of selection will be two-fold : first to isolate from among the foundation strains those which were superior from the beginning, and secondly, to improve the performance of the original strains by favouring their best components. (Lines 6 and 7 in Tables II to V). The intensity of selection can be correspondingly divided into two parts, first the difference between the mean of strains selected for propagation in the next year and the mean of all strains and secondly the difference between the mean of plants selected as parents and the mean of the selected strains to which they belong. (Lines 8 and 9 in Tables II to V).

TABLE II

Number of bolls per plant in Malvi selections

	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	38	25	15	11	8	5	5
Means.							
2. All strains examined . .	10·4	15·4	19·3	7·4	10·3	12·9	8·3
3. Selected strains .	11·6	17·5	19·4	7·3	12·0	12·9	..
4. Selected plants .	17·0	21·6	28·6	11·0	22·7	16·9	..
5. Five final selected strains .	13·8	17·6	20·6	7·4	12·0	12·9	8·3
Increments over previous year.							
6. All strains examined .	..	+5·0	+3·9	—11·9	+2·9	+2·6	—4·6
7. Selected strains over their own parents .	..	+3·8	+1·8	—12·0	+3·0	+0·9	—4·6
Selection differences.							
8. Selected strains from all strains	+1·2	+2·1	+0·1	—0·1	+1·7	0	..
9. Selected plants from selected strains .	+5·4	+4·1	+9·2	+3·7	+10·7	+4·0	..
10. Weather characteristics .	Late rains.	No late rains. Slight frost.	No late rains. Frost.	Serious late rains.	Serious late rains.	Late rains.	Late rains. Frost.

The data given for number of bolls per plant (Table II) are so strongly influenced by the incidence of late rains that no appreciable connection can be discovered between the selection intensities (lines 8 and 9) and the increments in the means in the following year (lines 6 and 7). That selection has been effective, however, is shown by the fact that in four out of the five comparable years, the mean number of bolls per plant was greater for the five strains finally selected than for all strains examined in the same years. It is clear that a group of strains with a higher mean-boll-number has been isolated, though it is not possible to say whether these strains have improved under the influence of selection.

TABLE III

Date of bursting of first boll, in Malvi selections (in days from October 1st)

	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	..	24	15	11	8	5	5
Means.							
2. All strains examined	..	43·6	20·9	40·0	55·0	33·8	29·3
3. Selected strains	22·4	44·0	20·5	39·7	55·4	33·8	..
4. Selected plants		43·4	21·4	38·6	52·5	32·5	..
5. Five final selected strains	22·9	44·7	21·6	38·7	55·4	33·8	29·3
Increments over previous year.							
6. All strains examined	..	..	—22·7	+19·1	+15·0	—21·2	—4·5
7. Selected strains over their own parents	..	..	—23·1	+19·5	+15·3	—21·7	—4·5
Selection differences.							
8. Selected strains from all strains	..	+0·4	—0·4	—0·3	+0·4	0	..
9. Selected plants from selected strains	..	—0·6	+0·9	—1·1	—2·9	—1·2	..
10. Weather characteristics	Late rains.	Very heavy early and August rains. No late rains.	No late rains.	Serious late rains.	Serious late rains.	Late rains.	Late rains.

NOTE.—Negative signs indicate earliness and positive signs lateness.

There is no evidence of any improvement in the data given in Table III for the date of bursting of the first boll, and in view of the small selection differences— not even always in the right direction—no improvement could reasonably be expected.

TABLE IV

Ginning percentage in Malvi selections

	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	38	25	11	10	5	5	2
Means.							
2. All strains examined . .	24·6	28·6	29·0	28·5	31·0	31·3	32·3
3. Selected strains .	25·0	28·9	29·7	30·8	31·0	31·0	..
4. Selected plants	26·4	31·3	29·0	33·2	33·4	32·7	..
5. Five final selected strains . .	28·4	31·5	32·3	30·8	31·0	31·3	..
Increments over previous year.							
6. All strains examined . .	..	+4·0	+0·4	—0·5	+2·5	+0·3	+1·3
7. Selected strains over their own parents . .	..	+3·6	+0·1	—1·2	+0·2	+0·3	+1·3
Selection differences.							
8. Selected strains from all strains	+0·4	+0·3	+0·7	+2·3	0	—0·3	..
9. Selected plants from selected strains . .	+1·4	+2·4	—0·7	+2·4	+2·4	+1·7	..

The ginning percentage data (Table IV) show a steady year-by-year improvement which can safely be ascribed to selection. One anomaly is evident. The increment of 1928-29 (all strains) over 1927-28 (all strains) was 4·0, whereas the total selection difference in 1927-28 is only 1·8. The increment is therefore considerably greater than can reasonably be ascribed to selection. Apart from this exception the yearly increments are roughly proportional to the total selection difference, and in 1930-31 there was a drop in mean ginning percentage (all strains) following a zero net selection difference in 1929-30. The five finally selected strains were in all four years superior to the mean of all strains, and within themselves they show no appreciable year-by-year improvement, indicating that most at least of the selective gain has been due to the isolation of strains superior to the average from the beginning, and not to improvement within strains.

TABLE V

Lint-length in Malvi selections (halo-length in mm.)

	1926-27	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	34	29+6	25	11	11	5	5	5
Means.								
2. All strains examined .	20·0	20·1	23·2	22·7	22·9	22·7	22·3	23·1
3. Selected strains	20·1	20·6	23·3	22·7	23·1	22·7	22·3	..
4 Selected plants	20·8	21·5	23·8	23·0	23·8	23·2	23·3	..
5. Five final selected strains . .	20·8	20·2	23·2	23·1	23·1	22·7	22·3	23·1
Increments over previous year.								
6. All strains examined . .	..	+0·1	+3·1	—0·5	+0·2	—0·2	—0·4	+0·8
7. Selected strains over their own parents . .	..	0	+2·6	—0·6	+0·2	—0·4	—0·4	+0·8
Selection differences.								
8. Selected strains from all strains	+0·1	+0·5	+0·1	0	+0·2	0	0	..
9. Selected plants from selected strains . .	+0·7	+0·9	+0·5	+0·3	+0·7	+0·5	+1·0	..

There were small positive selection differences in halo-length in all years (Table V). Apart from the large jump between 1927-28 and 1928-29 mentioned above, changes in mean halo-length from year to year were small, and there is no evidence of any upward trend which could be ascribed to the influence of selection. It is not possible to ascribe the difference between 1927-28 and 1928-29 means to the effect of selection, as it is much larger than the corresponding selection difference.

IV. DISCUSSION

On the whole changes which can reasonably be ascribed to the influence of selection are disappointingly small, although two very superior strains have in fact been isolated. Environmental influences appear to have masked a real selective effect on boll number. Improvement in earliness would not be expected with the negligible selection differences employed, even if environmental fluctuations had been less extreme. The halo-length results are the most disappointing. A steady, though small, selection difference was maintained throughout, and environmental influences were not serious, but no improvement ascribable to selection was obtained.

For the improvement of plant breeding technique it is most important to discover whether the potentialities of the original material were fully exploited. If the original genetic variance was so low that the actual improvement obtained was the maximum possible, no improvements in technique would affect the achievements of selection. If, however, considerable genetic variance existed in the original population and remained undetected by the selection methods adopted, the improvement of breeding technique may be expected to make a corresponding improvement in the achievements of selection.

In the 1934-35 season progeny-rows of the two finally selected strains, Malvi 1 and Malvi 9, were laid out in two experiments in randomised plots replicated ten times, each plot consisting of a single row of five plants. From the data obtained from these experiments it was possible to estimate the genetic variance for various characters existing between progenies of two strains self-fertilised for eight generations. Data are given in Tables VI and VII.

TABLE VI

Means, standard errors, and significance of differences in replicated progeny-rows of Malvi 1, 1934-35

Progeny numbers.	1	2	3	4	5	6	7	8	9	10	Per cent st. error	Signi-ficant diff.	Order of merit
Total yield of *kapas* in gms. per progeny.	558·9	629·1	531·1	627·2	535·0	474·0	641·7	498·0	629·3	660·8	14·9	...	10, 7, 9, 2, 4, 1, 5, 3, 8, 6
Total stand per progeny	41	45	43	47	33	38	39	36	45	45	7·1	8·34	4, 2, 9, 10, 3, 1, 7, 6, 8, 5
Total fruiting nodes per progeny.	2202	1931	1538	2021	1669	1882	2048	1837	2052	2089	10·5	...	1, 10, 9, 7, 4, 2, 6, 8, 5, 3
Mean ginning per cent per progeny.	27·8	30·1	28·5	31·9	27·3	27·3	27·1	27·1	27·7	27·8	1·8	1·47	4, 2, 3, 10, 1, 9, 5, 6, 7, 8
Mean vigour per progeny	—1·2	—1·5	—1·0	—0·9	—1·0	—1·1	—0·7	—1·0	—1·1	—1·2	18·7	...	2, 1, 10, 6, 9, 3, 5, 8, 4, 7
Mean lint-length in mms. per progeny.	22·9	22·9	22·4	22·6	22·7	23·2	23·3	23·1	22·9	22·6	0·99	...	7, 6, 8, 1, 2, 9, 5, 4, 10, 3
Mean node number per progeny.	11·54	10·13	11·29	10·81	10·50	11·60	11·87	11·78	12·12	12·05	2·1	0·67	2, 5, 4, 3, 1, 6, 8, 7, 10, 9

TABLE VII

Means, standard errors, and significance of differences in replicated families of Malvi 9, 1934-35

Family numbers.	13	15	17	19	20	St. error per cent for families	Sig. diff.	St. errors per cent for progenies	Families with progenies differing significantly	Remarks
Mean yield of *kapas* in gms. per family per plot	41·14	40·58	48·72	52·93	50·03	3·8	4·7	11·5	...	19, 20, 17 | 13, 15
Final mean stand per family per plot	4·17	4·38	4·33	4·55	4·70	3·5	0·38	5·3	13, 15, 17, 19, 20	20, 19 | 15, 17, 13
Mean ginning per cent per family	31·97	32·08	32·48	32·63	32·26	0·5	0·468	1·9	...	19, 17, 20, 15, 13
Mean fruiting nodes per family per plot	152·28	157·24	178·57	186·43	180·69	2·5	12·0	7·7	...	19, 20, 17 | 15, 13
Total vigour per family .	+9	—4	—56	—51	—50	35·8	30·8	90·0	15, 17, 19, 20	17, 19, 20 | 15, 13
Mean lint-length per family	21·04	21·28	21·28	21·17	21·89	0·55	0·33	1·5	...	20 | 17, 15, 19, 13
Mean node number per progeny	11·4	11·5	11·8	10·5	11·3	1·4	0·5	32·8	17, 19	19, 20 | 13, 15, 17
Percentage yield before frost per family per plot	63·9	65·0	59·0	60·0	58·5	2·9	5·0	8·8	...	15, 13 | 19, 17, 20

The ten progenies of Malvi 1 were from sister plants of the 1933-34 crop. Between them there were demonstrated significant differences in total stand, ginning percentage, and node number.

In Malvi 9, ten progenies were grown from each of five different families belonging to two different sub-strains. The fifty progenies were randomised. and replicated ten times, each progeny contributing ten single-row plots of five plants each. Significant differences between families were demonstrated for every character studied, even including vigour for which the standard error was as high as thirty-six per cent. Significant differences between progenies within families were demonstrated for final stand, vigour and node number. Yield of *kapas*, ginning percentage, fruiting nodes, vigour, lint-length and percentage of yield before frost are all characters directly affected by the selection practised, while final stand and node number are not. In the single family of Malvi 1 studied genetic variance remains for both the characters not influenced by the selection practised, and also for ginning percentage, in which selection has been most effective. In Malvi 9 genetic variance exists between families for all characters studied, and within families for the two not affected by selection, and also for vigour.

The genetic variance between families is an index of the genetic variance existing in the single plant of the 1928 progeny of Malvi 9 from which all families were descended, while the genetic variance existing between progenies of the same family in 1934-35 is an index of the genetic variance within the five plants from which they sprang in 1932. A comparison of the extent of genetic variance between and within families shows that a great deal was lost in the four years, 1928-32. This is presumably due to increasing homozygosis. It is abundantly clear that there was a large amount of genetic variance available in 1928 for characters on which selection was being practised. It was not exploited, and remained undetected until more sensitive methods were adopted in 1934-35. Much of it was lost from within families by increasing homozygosis in the intervening period, but sufficient remains to provide material for further selection in some characters, notably ginning percentage in Malvi 1, the character most successfully influenced by selection hitherto, and vigour in Malvi 9.

The method of randomisation and replication of progeny rows provides a measure, not only of the mean differences between progenies, but also of the errors to which these differences are subject, and enables the plant breeder not only to choose the best strains for further propagation, but also to decide with confidence whether their superiority is due to genetic or environmental causes. Its sensitiveness as a selection method is abundantly clear from the data presented in Tables VI and VII. If such potentialities for further improvement can be demonstrated in material on which selection has already been practised with great success, it is reasonable to expect that with unselected material the speed and extent of improvement will be greater.

There remains for consideration the question, to what is the improvement of the selected strains over the local due, if not to the selection method? There appears little doubt that it must be ascribed to that indefinable judgment of unmeasured characters, the necessity for which makes plant breeding as much an art as a science. It is to this plant-breeding sense that the great breeders of the past have owed their success. While it is hoped that this discussion makes it clear that greatly superior statistical weapons can now be placed in the breeder's hands, it should be emphasised that no improvement in statistical methods, however great, can absolve the breeder from the trouble of learning his material. He must know his plants as he knows his own brother if he is to exercise that judgment which is—and will remain—the secret of his success.

V. Summary

(1) The aims, methods, and achievements of selection in Malvi cotton are summarised.

(2) Two strains superior to the local mixture in all characters have been isolated and are being distributed.

(3) The methods and achievements of selection are analysed, and it is concluded that on the whole the changes ascribable to selection have been disappointingly small in spite of the superiority of the finally selected strains.

(4) Data from randomised, replicated progeny-row experiments on the selected strains are summarised and show clearly that the methods of selection adopted were not sufficiently critical to detect a large amount of genetic variance which was present in the material.

(5) The advantages for selection work of replication and randomisation of progeny rows are pointed out.

(6) The success achieved is ascribed primarily to the breeder's judgment and not to the selection methods. The paramount importance of the breeder's art is emphasised, and it is pointed out that no improvement in selection technique will free him from the need to gain that familiarity with his material which makes the successful exercise of his judgment possible.

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IDEAL SIZE AND SHAPE OF SUGARCANE EXPERIMENTAL PLOTS BASED UPON TONNAGE EXPERIMEMTS WITH Co. 205 AND Co. 213 CONDUCTED IN PUSA

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INTRODUCTION

The importance which the question of plot size and shape bears in relation to field experiments with different crops has now been realised by all agricultural workers. It has now become more than necessary to standardize for several experimental crops grown under different conditions such as irrigated or rain-fed, a suitable plot size and shape for the experimental plot which will reduce the standard error of the experiment to a practical minimum. Field experiments in other countries planned on modern lines of field technique have shown that the plot size and shape do exercise a considerable influence on the standard error of the experiment and that a particular size or shape of an experimental plot is always reached for a particular crop on a particular soil when the distribution of plot yields (subjected to the same treatment, other conditions of experiment remaining identical) can be ascribed to chance or chance alone. The question of economy in land and labour demands that the size of plot in the experiment should be as small as possible consistent with efficiency, and with a crop like sugarcane which is expensive to grow and occupies the land for a year, the need for fixing up a suitable plot size for experiments is acutely felt.

With such aims before us, the cutting experiments with Co. 205 and Co. 213, detailed subsequently, were undertaken to find out the most suitable plot size and shape for sugarcane experiments under unirrigated conditions. Up till now no work on this line has been done in India with sugarcane which is now one of the most important economic crops in India. The importance of an investigation of this nature to agricultural experimenters can, therefore, hardly be over-emp asised.

It is equally important to recognise the need for a correct statistical technique for problems of this kind, particularly as most of the agricultural farms in India are now making efforts to standardise the size and shape of their experimental plots.

I. TONNAGE EXPERIMENTS WITH Co. 205 IN 1932 AT PUSA

Materials and methods.—A tonnage experiment on sugarcane, Co. 205, unirrigated, was conducted in Harpur Jhilli in 1932. Forty-two rows of cane with a space of three feet between rows were selected and cut by sections, each section being 30 ft. 3 in. long. Thus the yield figures of plot sizes 30 ft. 3 in. × 3 ft. (*i.e.*, 1/480 acre each), numbering 840 such plots in all, were available for statistical analysis ; for convenience the data of yields of the first forty rows were also considered separately.

Experimental results.—The yields of several plot sizes were combined in all possible ways, and the coefficient of variation of each size was worked out (*i.e.*, the percentage ratio of standard deviation to mean) (the details of the method have been explained in subsequent pages).

TABLE I

Analysis of the data of tonnage experiments with Co. 205 sugarcane in 1932

Plot size	Area (acre)	Mean yield per plot (in lbs.)	S. D.	Coef. of variation $\left(=\frac{\text{S.D.}\times 100}{\text{mean}}\right)$	*Relative precision corrected for number of replications
I. (*a*) 30 ft. 3 in. × 3 ft., 42 rows.	1/480	90·7	31·55	34·8	100
30 ft. 3 in. × 3 ft., 40 rows.	1/480	92·65	30·55	32·9	107
II. (*a*) 30 ft. 3 in. × 6 ft., 40 rows.	1/240	185·3	46·9	25·3	91
30 ft. 3 in. × 6 ft., 42 rows.	1/240	181·4	47·4	26·1	89
(*b*) 60 ft. 6 in. × 3 ft., 42 rows.	1/240	181·4	53·9	29·7	69
60 ft. 6 in. × 3 ft., 40 rows.	1/240	185·3	51·8	28·0	74
III. (*a*) 60 ft. 6 in. × 6 ft., 40 rows.	1/120	370·6	83·2	22·5	58
60 ft. 6 in. × 6 ft., 42 rows.	1/120	362·8	89·6	24·7	49
(*b*) 121 ft. × 3 ft., 42 rows	1/120	362·8	92·4	25·5	47
121 ft. × 3 ft., 40 rows	1/120	370·6	85·26	23·0	55
(*c*) 30 ft. 3 in. × 12 ft., 40 rows.	1/120	370·6	72·2	19·5	76

*For the method of calculation of this column, see pages 700-701.

TABLE I—*contd.*

Plot size	Area acre)	Mean yield per plot (in lbs.)	S. D.	Coef. of variation ($=\frac{S.D. \times 100}{mean}$)	*Relative precision corrected for number of replications
IV. (*a*) 60 ft. 6 in. × 9 ft., 42 rows.	1/80	544·2	124·81	22·9	37
V. (*a*) 121 ft. × 6 ft., 40 rows	1/60	741·2	145·2	19·6	38
121 ft. × 6 ft., 42 rows	1/60	725·6	167·2	23·0	29
(*b*) 60 ft. 6 in. × 12 ft., 40 rows.	1/60	741·2	140·2	19·0	40
(*c*) 30 ft. 3 in. × 24 ft., 40 rows.	1/60	741·2	130·8	17·6	47
VI. (*a*) 60 ft. 6 in. × 18 ft., 42 rows.	1/40	1088·4	210·54	19·3	24
(*b*) 121 ft. × 9 ft., 42 rows	1/40	1088·4	220·2	20·2	25
VII. (*a*) 60 ft. 6 in. × 24 ft., 40 rows.	1/30	1482·4	279·5	18·85	22
(*b*) 121 ft. × 12 ft., 40 rows	1/30	1482·4	236·96	16·0	28
VIII. (*a*) 121 ft. × 18 ft., 42 rows	1/20	2176·4	382·32	17·6	16
IX. (*a*) 151 ft 3 in. × 18 ft., 40 rows.	1/15	2779·5	502·4	18·1	13
(*b*) 302 ft. 6 in. × 9 ft., 40 rows.	1/15	2779·5	501·06	18·0	13

The plot size giving the minimum coefficient of variation was considered to be a suitable size for an experimental plot.

The plot size with a minimum coefficient of variation in this case was found to be between 1/30 and 1/20 acre with dimensions 121 ft.×12 ft. and 121ft. × 18ft. respectively. The results also showed generally that as the shape of the plot got narrower, the coefficient of variation gradually diminished.

The chief defect of the experiment was found, however, to be that the soil variation between plot and plot or row and row was highly marked, as Table I of the coefficient of variation for different plot sizes, and of rapidly decreasing efficiency corrected for the number of replications, clearly shows. The coefficient of variation, however, went down from thirty-five per cent to sixteen per cent as the plot size increased, but in no case was it less than sixteen per cent which itself was indeed a very high figure with a corresponding precision of twenty-eight per cent. It was therefore decided that the experiment should be repeated next year, but with a more popular variety of cane Co. 213, on soils of fairly uniform fertility.

II. Tonnage experiments with Co. 213 in 1933 at Meghaul (Monghyr)

Co. 213 is recognised as the standard cane of the white sugar tract and represents probably the most generally grown cane crushed in sugar factories on the tract. All important cane experiments conducted at Pusa are laid down under this variety or Co. 210 and it was therefore felt advisable to conduct this experiment with Co. 213.

General description and history of Co. 213

Detailed description and history of Co. 213 have been given by Venkatraman and Vittal Rao [1928].

This cane, which has attained such universal success in North India was bred by Dr. C. A. Barber at Coimbatore and was first sent by him to Pusa in 1918-19 together with a lot of other seedlings. It was tested here in small plots for two years and then selected together with Co. 210 and Co. 214 for the first mill trial by the Secretary, Sugar Bureau, Pusa in December, 1922 [Sayer, 1923]. The test was conducted at Ryam, one of the sugar factories of Messrs. Begg Sutherland & Co. near Darbhanga by kind courtesy of the firm.

TABLE II

Chemical analysis of the first mill juice

Date of crushing	Variety	Brix	Sucrose	Purity
27th December 1922 . .	Co. 213	17·63	14·52	82·86

The fibre contents in Co. 213 and Co. 210 were found to be 16·84 and 15·66 per cent respectively lower than Co. 214. The poor sucrose percentage of Co. 213 in December is accounted for by the fact that it is a late-ripening cane, reaching its maximum at the end of January. The following analysis of juice was given by the Imperial Agricultural Chemist, Pusa, in 1933 :—

TABLE III

Chemical analysis of Co. 213 in January 1933

Variety	Date of analysis	Average weight of cane in lb.	In juice		
			Juice	Sucrose	Purity
Co. 213	21st Jan. 1933	1·68	Per cent 63·43	Per cent 17·39	Per cent 86·83

Having proved its high value in the mill trial and having displayed a remarkable tonnage capacity and excellent agricultural habit on an estate scale, its cultivation was extended over a larger area in 1922-23 by the Secretary, Sugar Bureau, in order to supply seed to cane growers of the Province. The cane is now cultivated in all provinces of North India; it occupies the first place as regards area under cultivation, in Bihar, Bengal and the United Provinces. It is also grown on a fairly large area in the Punjab, Madras, Bombay and Assam. The average tonnage of this cane on the Pusa Farm has been about 600 maunds (22·0 tons) per acre.

Materials and methods

The experiment was conducted at Meghaul, District Monghyr, on a six acre plot of Co. 213 cane very kindly made available for the purpose by Mr. C. Atkins, Manager, Dowlatpore Agricultural Concern, without whose valuable assistance it would have been impossible to conduct the experiment.

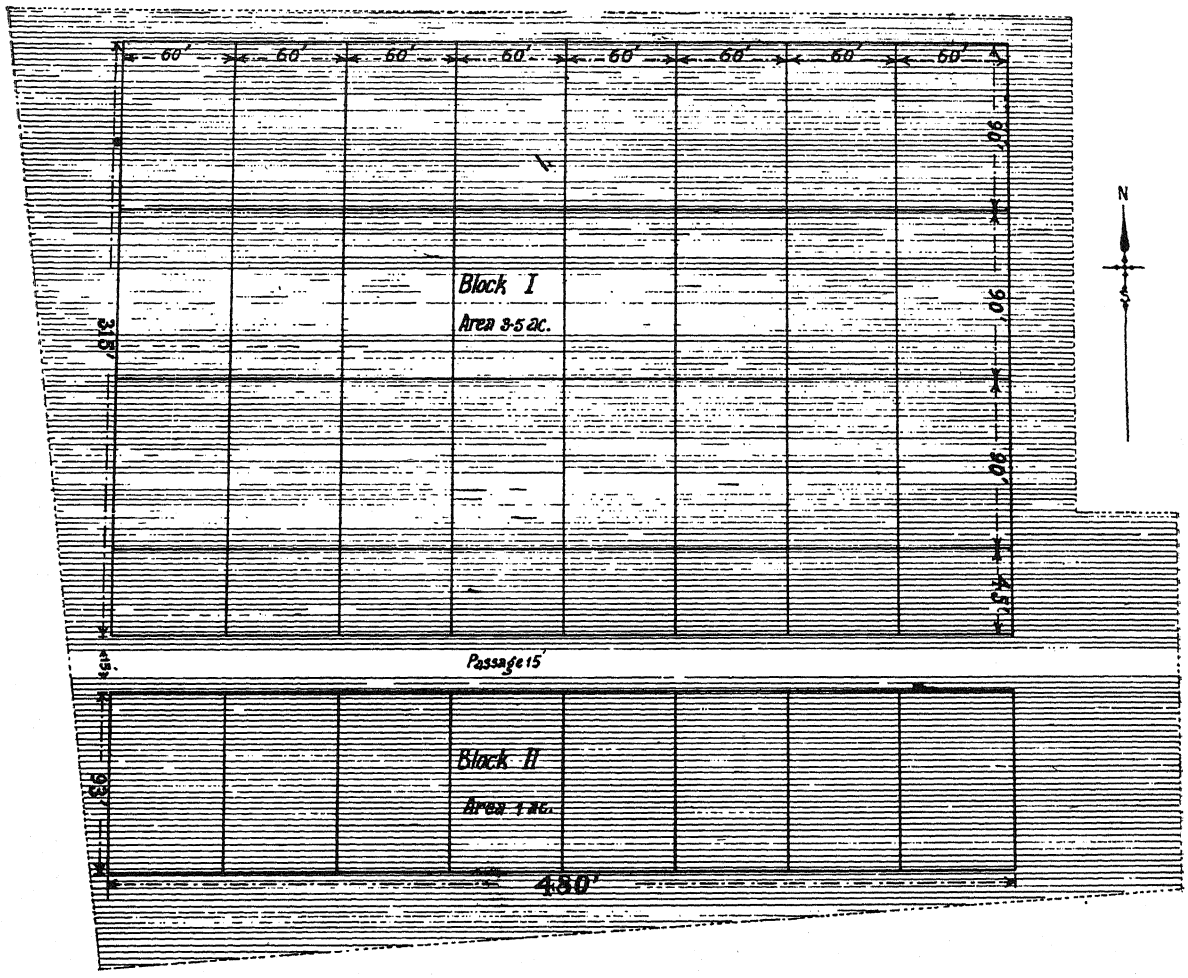
The land was under a regular sugarcane rotation in previous years; cultural methods, manuring and other operations were uniform all over the area. The soil throughout the field was a good class of cane loam—a typical *sumbi*. The field was planted on the 12th February, 1932; the setts were planted eye to eye in rows three feet apart with six feet of the ends doubled. Manuring was done at the rate of 400 maunds (14.7 tons) farmyard manure per acre which was purchased at the rate of one anna per maund locally. The operation of earthing up the rows was duplicated, once before and once after the break of monsoon. The cane was not irrigated, and the record of rainfall for the year of its growth is given below :—

	Inches
1st January to 31st May	*Nil*
June	5·49
July	4·49
August	9·18
September	5·34
October	0·99
November	3·66
Total	29·15
Average annual rainfall	47

The crop presented a very uniform growth and there was very little lodging.

The cutting of the canes was started on the 12th February, 1933. Before the commencement of the experiment, a passage of fifteen feet wide had been cut through the field to facilitate inspection. The crop was thus located in two blocks (Blocks I and II) separated by a temporary road. These blocks were

PLATE XLI



Plan of Co. 213 sugarcane field at Meghaul, Monghyr, showing lay-out of the cutting experiment.

trimmed on all sides by removing the border crop ranging from nine to thirty feet in width and shaped into two perfect rectangular blocks. The blocks then measured 480 ft. × 315 ft. (three acres and a half) and 480 ft. × 93 ft. (one acre) as shown in the plan (Plate XLI).

After the removal of the border crop, each row measured 480 feet in length and there were 136 such rows, each three feet apart, running east to west in an entire area of four acres and a half. The next step was to divide each row into eight equal sections; the ultimate plot obtained in this way consisted of one row a-piece, and this time it was 60 ft. × 3 ft. = 1/242 acre. These plots were cut, stripped and weighed separately guarding against all possible sources of error. The total number of weighings carried out in this way was 1,088, and it took sixteen days to finish the entire work. The weight of canes from each plot was originally recorded in pounds on two platform weighing machines which proved very useful for the purpose. Appendix gives the yields in pounds of the cuttings in several rows.

The crop cut out at an average weight of 1,000 maunds (36·7 tons) per acre, thus proving that such tonnages can be obtained in a year of short rainfall with proper manuring and soil management without any irrigation. It may also be noted that no artificials of any kind were applied to the crop.

Statistical methods adopted and results

Instead of dealing with the entire field, four sub-blocks of thirty rows each, as shown below, were taken out for the purpose of this statistical study :—

	Rows
Sub-block I (Block I)	1—30
,, II (,,)	31—60
,, III (,,)	61—90
(The other rows 15 in number were omitted.)	
Sub-block IV (Block II)	106—135
(The last row was omitted.)	

The omission of the rows was with the object of securing compact blocks of thirty rows each.

The soil variation in this experiment was found much less marked than in the case of the experiment last year, as the coefficient of variation had in no case exceeded 10·4 per cent. Though we could possibly have all combinations of plot sizes varying from 1/242 acre to 120/242 acre, the plot sizes beyond 16/242 acre were omitted as the frequency was small. We are thus left for each sub-block with thirteen different plot sizes as shown in Tables IV-VII ; the plot sizes with frequencies of multiples of five alone being considered for reasons explained in page 696.

TABLE IV

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{S.D. \times 100}{Mean}\right)$	S. E. of V.
I	1×1	60 ft. × 3 ft.	20	1/242	240	340·8	31·4	9·2	0·4234
	1×2	60 ft. × 6 ft.	10	2/242	120	681·6	41·2	6·0	0·3925
	2×1	120 ft. × 9 ft.	40	2/242	120	681·6	45·5	6·7	0·4354
	1×3	60 ft. × 9 ft.	6·7	3/242	80	1,022·4	61·4	6·0	0·4760
	4×1	240 ft. × 3 ft.	80	4/242	60	1,363·2	71·0	5·2	0·4760
	2×2	120 ft. × 6 ft.	20	4/242	60	1,363·2	81·64	6·0	0·5497
	1×6	60 ft. × 18 ft.	3·3	6/242	40	2,044·8	100·9	4·9	0·5491
	2×3	120 ft. × 9 ft.	13·3	6/242	40	2,044·8	98·6	4·8	0·5378
	8×1	480 ft. × 3 ft.	160	8/242	30	2,726·4	104·7	3·8	0·4914
	4×2	240 ft. × 6 ft.	40	8/242	30	2,726·4	129·5	4·7	0·6082
	4×3	240 ft. × 9 ft.	26·7	12/242	20	4,089·6	178·3	4·4	0·6971
	2×6	120 ft. × 18 ft.	6·7	12/242	20	4,089·6	179·6	4·4	0·6971
	8×2	480 ft. × 6 ft.	80	16/242	15	5,452·8	212·8	3·9	0·7131

TABLE V

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{S.D. \times 100}{Mean}\right)$	S. E. of V.
II	1×1	60 ft. × 3 ft.	20	1/242	240	338·6	28·6	8·4	0·3861
	1×2	60 ft. × 6 ft.	10	2/242	120	677·2	40·2	5·9	0·3822
	2×1	120 ft. × 3 ft.	40	2/242	120	677·2	41·5	6·1	0·3952
	1×3	60 ft. × 9 ft.	6·7	3/242	80	1,015·8	51·1	5·0	0·3962
	4×1	240 ft. × 3 ft.	80	4/242	60	1,354·4	68·4	5·0	0·4575
	2×2	120 ft. × 6 ft.	20	4/242	60	1,354·4	63·91	4·7	0·4300
	1×6	60 ft. × 18 ft.	3·3	6/242	40	2,031·6	85·1	4·2	0·4704
	2×3	120 ft. × 9 ft.	13·3	6/242	40	2,031·6	90·4	4·5	0·5041
	8×1	480 ft × 3 ft.	160	8/242	30	2,708·8	110·7	4·1	0·6602
	4×2	240 ft. × 6 ft.	40	8/242	30	2,708·8	106·5	3·9	0·5043
	4×3	240 ft. × 9 ft.	26·7	12/242	20	4,063·2	116·7	2·9	0·4589
	2×6	120 ft. × 18 ft.	6·7	12/242	20	4,063·2	141·0	3·5	0·5541
	8×2	480 ft. × 6 ft.	80	16/242	15	5,417·6	136·4	2·5	0·4568

TABLE VI

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{\text{S. D.} \times 100}{\text{Mean}}\right)$	S. E. of V.
III	1×1	60 ft.× 3 ft.	20	1/242	240	330·2	31·45	9·5	0·4375
	1×2	60 ft.× 6 ft.	10	2/242	120	660·4	47·74	7·2	0·4671
	2×1	120 ft.× 3 ft.	40	2/242	120	660·4	48·74	7·4	0·4803
	1×3	60 ft.× 9 ft.	6·7	3/242	80	990·6	63·25	6·4	0·5081
	4×1	240 ft.× 3 ft.	80	4/242	60	1,320·8	81·39	6·2	0·5682
	2×2	120 ft.× 6 ft.	20	4/242	60	1,320·8	80·37	6·1	0·5590
	1×6	60 ft.×18 ft.	3·3	6/242	40	1,981·2	86·99	4·4	0·4929
	2×3	120 ft.× 9 ft.	13·3	6/242	40	1,981·2	107·85	5·4	0·6055
	8×1	480 ft.× 3 ft.	160	8/242	30	2,641·6	130·54	4·9	0·6342
	4×2	240 ft.× 6 ft.	40	8/242	30	2,641·6	136·55	5·2	0·6732
	4×3	240 ft.× 9 ft.	26·7	12/242	20	3,962·4	199·36	5·0	0·7924
	2×6	120 ft.×18 ft.	6·7	12/242	20	3,962·4	147·47	3·7	0·5858
	8×2	480 ft.× 6 ft.	80	16/242	15	5,283·2	247·27	4·7	0·8600

TABLE VII

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{\text{S. D.} \times 100}{\text{Mean}}\right)$	S. E. of V.
IV	1×1	60 ft.× 3 ft.	20	1/242	240	295·0	27·6	9·4	0·4375
	1×2	60 ft.× 6 ft.	10	2/242	120	590·0	49·6	8·4	0·4671
	2×1	120 ft.× 3 ft.	40	2/242	120	590·0	41·1	7·0	0·4803
	1×3	60 ft.× 9 ft.	6·7	3/242	80	885·0	76·4	8·6	0·5081
	4×1	240 ft.× 3 ft.	80	4/242	60	1,180·0	62·5	5·3	0·5682
	2×2	120 ft.× 6 ft.	20	4/242	60	1,180·0	123·2	10·4	0·5590
	1×6	60 ft.×18 ft.	3·3	6/242	40	1,770·0	160·4	9·1	0·4929
	2×3	120 ft.× 9 ft.	13·3	6/242	40	1,770·0	117·8	6·7	0·6055
	8×1	480 ft.× 3 ft.	160	8/242	30	2,360·0	104·5	4·4	0·6342
	4×2	240 ft.× 6 ft.	40	8/242	30	2,360·0	102·4	4·3	0·6732
	4×3	240 ft.× 9 ft.	26·7	12/242	20	3,540·0	195·8	5·5	0·7924
	2×6	120 ft.×18 ft.	6·7	12/242	20	3,540·0	275·1	7·8	0·5858
	8×2	480 ft.× 6 ft.	80	16/242	15	4,420·0	174·0	3·9	0·8600

Differences in yields in different sub-blocks

The distributions of yields of different plot-sizes in all the four sub-blocks were found generally * to be normal. The exact distributions, and the validity of Fisher's z-test as applied to these data are being tested (in a separate paper to follow). A large number of possible arrangements are taken and tested for agreement between the actual and theoretical values. In the present case, both the mean yields and the coefficients of variation were in all cases found to be very high as compared to their respective standard errors, showing a high degree of accuracy for these statistical coefficients. Comparing the mean yields of plots in the several sub-blocks, it is seen from the tables that the mean yield remains almost the same in the first three sub-blocks, while sub-block IV is found significantly inferior in fertility to the other sub-blocks. The analysis of variance of yields for the plot size 120 ft. × 18 ft. = 12/242 acre for the several sub-blocks is given in Tables VIII (*a*, *b*, *c*).

Table VIII (*a*)

Analysis of variance of the yields of sub-blocks (I to III) for plot size 120 ft. × 18 ft. = 12/242 acre

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
Between blocks . .	2	137,580	68,790	0·4347
Within blocks . . .	57	1,644,123	28,844	Less than 5 per cent value of 'z' and hence not significant.
Total .	59	1,781,703	..	

Table VIII (*b*)

Analysis of variance of the yields of sub-blocks (I to IV) for plot size 120 ft. × 18 ft. = 12/242 acre

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
Between blocks . . .	3	3,894,082	1,298,027	1.6496
Within blocks . . .	76	3,641,003	47,921	Greater than one per cent value of 'z' and hence highly significant.
Total .	79	7,535,085	..	

* In the case of plots whose number in each sub-block is less than 20, the exact distribution is not ascertainable.

TABLE VIII (*c*)

Mean yields of sub-blocks in lbs.

Sub-blocks	I	II	III	IV	S. E. of mean of 20 plots	Critical difference (5 per cent level)
Mean yields . .	4,090	4,063	3,962	3540	48·95	137·74

(I = II = III > IV).

In the case of the first three sub-blocks, '*z*'-test shows no significant difference in yields between 'blocks', and 'within blocks' for this particular plot size, while by combining all the four sub-blocks, the variance due to 'blocks' is found to give a significant difference from that due to 'within blocks'. Comparisons between sub-blocks on the basis of '*t*'-test bring out no significant difference between the first three sub-blocks and a significant difference between either of them and the fourth sub-block. It is, therefore, considered necessary to treat the fourth sub-block as distinct from the other sub-blocks, for the purpose of this study.

SOIL VARIATION IN THE SEVERAL SUB-BLOCKS

To examine the differences in plot yields in the individual sub-blocks, percentage deviations of yields for the plot-size 1 cut × 3 rows (= 3/242 acre) from the mean yield of the sub-block have been calculated, and are given in Table IX Plots with positive and negative deviations exceeding ten per cent (*i.e.*, patchy areas) are shown in block type. In sub-block I, of the eighty plots there are only eight patchy plots, three of which occur in the first row and four in the fifth column. In sub-block II the number of patchy areas is almost the same, but it seems more homogeneous than the first sub-block. Sub-block III contains a slightly larger number of patchy areas than the first two sub-blocks. Sub-block IV is marked by an appreciable number of patches with the fifth and sixth columns containing a large number of high-yielding plots. Analysis of variance between cuts and rows for the several sub-blocks is given in the Table X. It is interesting to see that in all the sub-blocks there is no significant difference in variation between 'cuts' and 'rows'. But in sub-block I, the variations in cuts and rows are both significant, in sub-block II there is a significant variation in rows only, in sub-block III the variations in both cuts and rows are not significant, and in sub-block IV both the cuts and rows have significant variations. Besides, the figures of percentage variations in the plot-yields indicate no systematic change in fertility in the several sub-blocks, nor could any competition between the plots be detected. Any plan of lay-out under conditions of fertility in any of these sub-blocks should therefore take into account the trend of variation indicated above.

TABLE IX

Percentage deviations in plot-yields from the mean yield of sub-block (plot size 1 cut × 3 rows = 3/242 acre)

	Rows	Cuts						Total
				Sub-block I				
	—7	+6	+6	—15	—16	—11	—2	+5	—34
	—1	0	—1	—5	—23	0	+5	+3	—22
	+3	+7	+9	+8	—1	+9	+10	+8	+53
	—4	+6	+4	+1	+4	0	+3	0	+14
	+8	—1	+8	+13	+2	+3	+3	—4	+32
	+2	+1	+13	—5	—5	—1	+8	+10	+23
	—1	0	—1	+4	—8	—4	+3	+6	—1
	+3	+3	+2	—5	—8	—6	+4	+6	—1
	—6	+3	+2	—6	—14	—1	+1	—1	—22
	—6	+3	+8	—3	—19	—7	—4	—4	—32
Total .	—9	+28	+50	—13	—88	—18	+31	+29	
				Sub-block II				
	+3	+4	+12	—2	—3	—13	+4	+3	+8
	+1	+8	+9	+9	—3	..	+3	+1	+28
	—10	+2	+8	+5	—3	—5	—3	—5	—11
	..	+5	—6	—2	+5	+5	+4	+7	+18
	+2	+6	—5	+4	+7	—3	—3	—5	+3
	+1	—13	—1	..	+5	—1	+3	+9	+3
	+3	+1	—3	+6	—2	—1	—3	+5	+6
	+2	—7	—15	+3	..	—8	—16	—3	—44
	+10	—10	—4	1	—13	..	—3	+4	—16
	—5	—8	+1	—13	—9	—1	—2	+5	—32
Total .	+7	—12	—4	+10	—16	—27	—16	+21	
				Sub-block III				
	—5	—3	+1	—5	—23	—19	—9	—2	—65
	—2	—7	+4	+6	—10	—1	+3	+6	—1
	+3	—1	+5	+8	+6	+11	+4	+1	+37
	—5	—4	+2	—2	—0	+5	+7	+6	+9
	—1	—9	—13	+1	—6	—1	—3	+4	—28
	—13	+8	—1	—2	—5	—5	+5	+2	—11
	—8	+11	+3	+11	—2	—1	+7	+12	+33
	+6	—1	—6	+4	+7	+7	+10	+7	+34
	—3	—10	—4	+3	—2	+4	—1	—5	—18
	—8	—5	+3	+1	+14	+3	+4	—4	+8
Total .	—36	—21	—6	+25	—21	+1	+27	+27	
				Sub-block IV				
	—4	—9	—7	+5	+31	+15	—4	—9	+18
	—7	—8	+9	+5	+18	+4	—8	+4	+17
	+13	+3	+3	+4	+6	+19	..	+13	+61
	—1	—14	—7	+3	+12	+28	—1	+17	+37
	+5	—20	—6	+12	+15	+17	+2	+11	+36
	—13	—29	—2	+14	+9	+12	+1	—4	—12
	+5	—10	—4	+5	+10	+18	—4	—13	+7
	+1	—10	—10	..	—3	+7	—1	—8	—24
	+3	—16	—6	—7	—18	—7	+1	—1	—51
	+4	—26	—24	..	—23	—2	—10	+10	—71
Total .	+6	—129	—54	+41	+57	+111	—24	+20	

TABLE X

Analysis of Variance

[*Based on data in Table IX*]

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
Sub-block I				
Cuts	7	4,046	578·0	(1) 1·017 Cuts and error.
Rows	9	1,922	213·6	(2) 0·520 Rows and error.
Error	63	4,884	75·5	
Total .	79	10,852		
Variations in 'Cuts' and 'Rows' are both significant as compared to error.				
Sun-block II				
Cuts	7	426	60·9	(1) 0·2933 Cuts and error.
Rows	9	1,162	129·1	(2) 0·6690 Rows and error.
Error	63	2,134	33·87	
Total .	79	3,722		
Variation in 'Cuts' is not significant as compared to error. Variation in 'Rows' is significant.				
Sub-block III				
Cuts	7	182	26·0	(1) 0·3143 Cuts and error.
Rows	9	553	61·4	(2) 0·2725 Rows and error.
Error	63	2,261	35·6	
Total .	79	2,996		
Variation in 'Cuts' and 'Rows' are both not significant.				
Sub-block IV				
Cuts	7	1,339	191·3	(1) 1·0441 Cuts and error.
Rows	9	962	106·9	(2) 0·7533 Rows and error.
Error	63	1,494	23·7	
Total .	79	3,795		
Variation in 'Cuts' and 'Rows' are both significant.				

Explanation of Tables IV-VII

Tables IV to VII are sufficiently self-explanatory. The standard deviation for each plot size in each sub-block is worked out by Fisher's analysis of variance after elimination of *division variance (*i.e.*, variance due to soil heterogeneity) on the assumption of five plots in each division. This assumption is made with a view to eliminate soil variance, while the same number of plots for every arrangement of plot size will ensure the same number of treatments in each block, in this case assumed to be five. For convenience, the case of plot sizes whose number in each sub-block is a multiple of five, alone has been considered. The arrangement of plots in each sub-block is in all cases rectangular. The method of analysis for working out the standard deviation is illustrated below :—

Sub-block I

Plot size 120 ft. × 18 ft. = 12/242 acre.

Variance due to	D. F.	Sum of squares	Mean Square	$\frac{1}{2}$ log (M. S	z
Between divisions . .	3	377324·1	125774·7	5·8712	
Within divisions . .	16	516098·0	32256·1	5·1907	0·6805
Total .	19	893422·1			

Five per cent z (for $n_1 = 3$ and $n_2 = 16$) = 0·5876

'z'—test in this case shows a significant difference between 'divisions' and 'within divisions'. Standard deviation per plot is the square root of 32256·1 *i.e.* 179·6. On this basis, the coefficient of variation (*i.e.* $\frac{\text{S. D} \times 100}{\text{Mean}}$) is then calculated. The standard error of the coefficient of variation has been calculated on the basis of the well-known formula :—

$$\text{S. E. of C. V.} = \sqrt{\frac{V}{2n}} \sqrt{\frac{}{r}} \left[1 + 2\left(\frac{V}{100}\right)^2\right],$$

where V is the coefficient of variation.

Table XI shows that by increasing the width or length of the plot the coefficient of variation rapidly goes down. Taking sub-blocks I—III, the series giving low coefficients of variation are :—2 rows × 8 cuts, 3 rows × 4 cuts and 6 rows × 2 cuts ; and the averages for sub-blocks I—IV, also give the same plot-sizes. But since the plot should have a sufficient number of rows to allow for border effect and convenience of cultivation, of the various plot-sizes, 6 rows × 2 cuts (*i.e.*, 12/242 acre) seems desirable.

*In this paper, 'division' is used in the sense of common usage 'block', not to confound with 'block' and 'sub-block' already used in the paper.

TABLE XI

Average coefficient of variation for different plot-sizes and shapes

Length of cut (60 ft.)	Rows (between row to row 3 feet)			
	1	2	3	6
	(*1*) *Sub-blocks I—III*			
1	9·0	6·4	5·8	4·5
2	6·7	5·6	4·9	3·9
4	5·5	4·6	4·1	
8	4·3	3·7		
	(*2*) *Sub-blocks I—IV*			
1	9·1	6·9	6·5	5·7
2	6·8	6·8	5·4	4·9
4	5·4	4·6	4·5	
8	4·3	3·8		

TABLE XII

Average coefficient of variation from several sub-blocks for different plot-sizes

Plot size (acreage)	Sub-blocks I—III	Sub-blocks I—IV
1/242	9·0	9·1
2/242	6·4	6·9
	6·7	6·8
3/242	5·8	6·5
4/242	5·5	5·4
	5·6	6·8
6/242	4·5	5·7
	4·9	5·4
8/242	4·3	4·3
	4·6	4·6
12/242	4·1	4·5
	3·9	4·9
16/242	3·7	3·8

Table XII and Fig. 1 emphasise the same point that the cofficient of variation diminishes gradually till a plot-size 12/242 to 16/242 of an acre is reached. This decrease in the coefficient of variation with increased size of plots is consistent with observations on other crops in other countries. The average coefficient of variation for the size 16/242 acre is 3·7 for the first three sub-blocks, and 3·8 for all the four sub-blocks taken together, and for the size 12/242 of an acre the averages are 3·9 and 4·5 respectively. In the same graph, theoretical coefficients of varia-

tion for different plot-sizes are also shown, and it is clear that the reduction in the actual coefficient of variation as plot-size increases is found less than that based on theoretical considerations. This indicates the existence of a correlation between the plots, and this point is examined in a subsequent para.

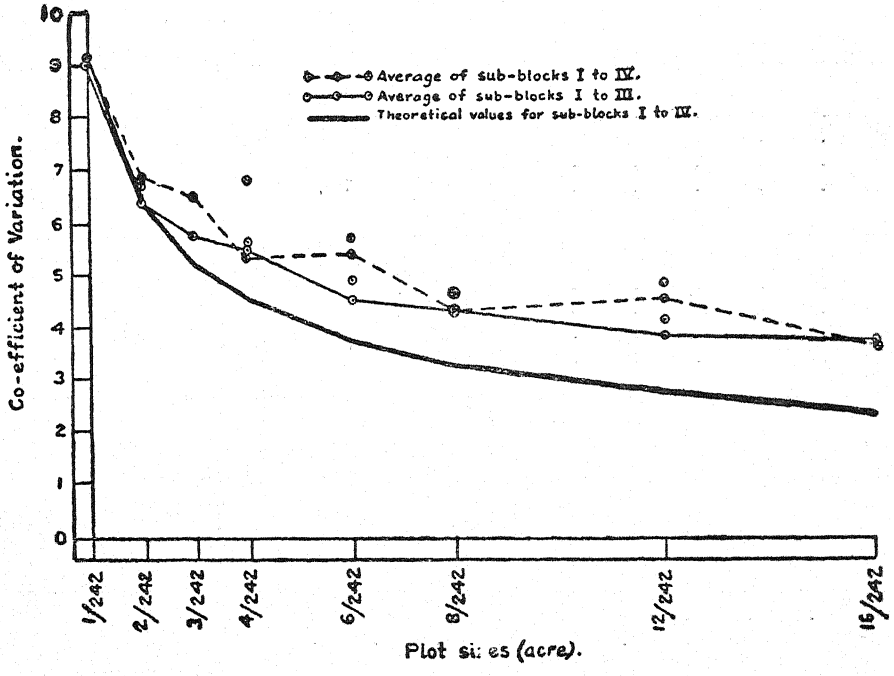


Fig. 1 (*vide* Table XII). The relation between coefficient of variation and size of plot.

(Stray points indicate different shapes for the same size.)

CHANGES IN THE COEFFICIENT OF VARIATION AS PLOT SHAPE CHANGES

Table XIII and Fig. 2 indicate how the coefficient of variation changes as the shape changes *i.e.*, as the ratio of length to width of the plot increases.

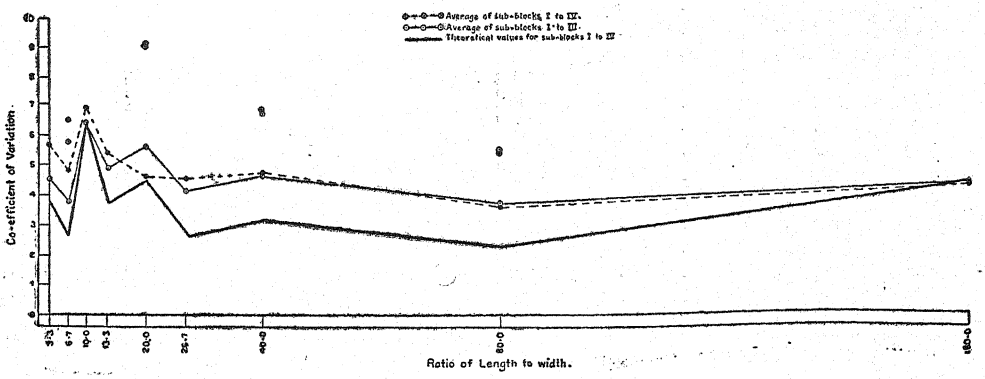


Fig. 2 (*vide* Table XIII). The relation between coefficient of variation and ratio of length to width of plot.

(Stray points indicate smaller sizes for the same shape.)

It is interesting to see that in the several sub-blocks, the coefficient of variation increases till the ratio 10 is reached ; then it shows a definite decline between the ratios 10 to 20 and thereafter has a fall, though not markedly, beyond ratio 20. The peaks at the ratios 10 and 20 in the graph are associated with very small plot-sizes. Excluding these two peaks, the smallest values centre round the ratios 6·7, 26·7 and 80. Leaving out of account the ratios 26·7 and 80 for the very good reason that the number of rows to be dealt with in such cases is very small—too small for making due allowance for border effect—the experimental plots, the ratio of whose length to width is about 7 : 1, seem to give low coefficient of variation, as compared to plots with lower ratios. The plot-size corresponding to this ratio is 12/242 of an acre. This emphasises the need for a long narrow experimental plot for sugarcane of about 12/242 of an acre in area.

TABLE XIII

Coefficient of variation for different plot shapes

Ratio of length to width	Sub-block I	Sub-block II	Sub-block III	Sub-block IV	Average for sub-blocks I—III	Average for sub-blocks I—IV
3·3 (6/242 acre) . .	4·9	4·2	4.4	9·1	4·5	5. 7
6·7 (3/242 „) . .	6·0	5·0	6.4	8·6	5·8	6. 5
6·7 (12/242 „) .	4·4	3·5	3.7	7·8	3.9	4·9
10·0 (2/242 „) .	6·0	5·9	7.2	8·4	6.4	6·9
13·3 (6/242) . .	4·8	4·5	5·4	6·7	4·9	5·4
20·0 (1/242 „) . .	9·2	8·4	9·5	9·4	9·0	9·1
20·0 (4/242 „) .	6·0	4·7	6·1	10·4	5·6	6·8
26·7 (12/242 „) .	4·4	2·9	5·0	5·5	4·1	4·5
40·0 (2/242 „) . .	6·7	6·1	7·4	7·0	6·7	6·8
40·0 (8/242 „) . .	4·7	3·9	5·2	4·3	4·6	4·6
80·0 (4/242 „) . .	5·2	5·0	6·2	5·3	5·5	5·4
80·0 (16/242 „) . .	3·9	2·5	4·7	3·9	3·7	3·8
160·0 (8/242 „) . .	3·8	4·1	4·9	4·4	4·3	4·3

TABLE XIV

Variances for plots varying in size and shape

Length of cut 60 ft.	Rows 1	2	3	6
	Average of sub-blocks I—III			
1	932	1,863	3,463	8,339
2	2,021	5,738	9,845	24,230
4	5,449	15,590	28,390	
8	13,415	41,682		
	Average of sub-blocks I—IV			
1	889	2,012	4,056	12,680
2	1,963	8,098	10,853	37,099
4	5,063	14,314	30,877	
8	12,792	38,830		

RELATIVE PRECISION OF DIFFERENT PLOT SIZES AND SHAPES

We have so far compared the different plot-sizes and shapes on the basis of their coefficient of variation, that is, on the basis of their standard deviations corrected for their means or for their plot-sizes. But the relative plot-precisionof

one plot size to another can be judged on the basis of their variances ; thus if one plot-size gives a variance A, and another a variance B, the relative precision of the first to the second is B/A. A plot size, however, has to satisfy other considerations, such as the number of replications possible with that size in an experimental field of a given extent. Modern methods of experimentation insist upon a sufficient number of replications and upon particular modes of arrangement. Replication in an experiment secures an error as low as possible and ensures a valid estimate of it. Therefore, in choosing one plot-size to another, our object should be to choose one with not only a lower variance, but one which would permit of proportionately larger number of replications in a field of a given extent. Thus, if the first size be n times the second with reference to a field of a given extent, the former can permit of only $\frac{1}{n}$ times the number of replications which the latter can admit, the relative precision of the first plot to the second (on the basis of variances A and B respectively) corrected for the number of replications will then be Bn/A.

Table XIV gives the average variances for different plot-sizes and Table XV their relative precision, assuming the precision of the smallest plot size to be 100.

TABLE XV

Relative precision of different plot-sizes corrected for replications

Plot acreage	Relative precision Blocks I—III	Relative precision Blocks I—IV
1/242 (1 cut × 1 row)	100·0	100·0
2/242 (1 cut × 2 rows)	100·0	88·4
2/242 (2 cuts × 1 row)	92·2	90·6
3/242 (1 cut × 3 rows)	80·7	65·8
4/242 (4 cuts × 1 row)	68·4	70·2
(2 cuts × 2 rows)	65·0	41·9
6/242 (1 cut × 6 rows)	67·1	42·1
(2 cuts × 3 rows)	58·9	49·2
8/242 (8 cuts × 1 row)	55·6	55·6
(4 cuts × 2 rows)	47·8	50·0
12/242 (2 cuts × 6 rows)	46·1	28·8
(4 cuts × 3 rows)	39·4	34·6
16/242 (8 cuts × 2 rows)	36·0	36·7

It is clear that on the above criterion of efficiency the smallest plot-size is the most desirable and that the relative precision decreases from 100 to 36 as the plot-size increases from 1/242 of an acre to 16/242 of an acre. The fall in efficiency is marked till we reach 8/242 of an acre, and thereafter between 8/242 and 16/242 of an acre the fall is not so rapid. Though the smallest plot-size available is the most efficient in view of a very large number of replications which it can permit,

still from the point of view of cultivation and to take account of border effect an irreducible minimum number of rows in a plot seems necessary.

Again between plots of the same size and different shapes, the differences in efficiency are not very marked, though generally speaking long narrow plots have, for all practical purposes given very good results.

DIVISION VARIANCE (*i.e.*, IN USUAL LANGUAGE BLOCK VARIANCE) *vs.* PLOT VARIANCE

We have seen from the present data that as plot-size increases, the coefficient of variation rapidly goes down. From general considerations, it is clear that with a very small plot-size, the variance between the plots is very small and that as the plot-size increases, the plot variance also increases owing to inclusion in each plot of differences in fertility obtaining in the field. Thus in any block arrangement, with small-sized plots, a larger portion of block variance is eliminated leaving a small plot variance, and with the increase in the size of plot beyond a certain stage, the elimination of block variance is not so effective, and the plot variance becomes large. If A be the block variance and B the plot variance, in fixing a suitable plot size we are seeking one after elimination of A giving as small a value as possible for B. In the case of a plot-size, where $B > A$ significantly, the lay-out fails in eliminating effectively the block variance, and the plot-size in such a case should be reduced. When B is small and A appreciable enough, the lay-out may be considered satisfactory; for in such a case while block-elimination is successful the plots will represent a random and sporadic variation in each block. An ideal lay-out is the one where the block-size should represent a sample of the general fertility in the field, and the plot-size a random variation giving a low standard error.

In the present case, from Table XVI it is seen that as plot-size continuously increases, the block arrangement is successful in eliminating an appreciable portion of division variance. When the plot size is 1/242 acre, the percentage of the sum of squares due to divisions to total sum of squares is between twenty-eight and thirty-six per cent in the first three sub-blocks and sixty-four per cent in the fourth sub-block. As plot size increases the variance due to divisions continuously increases and so also the plot variance. In no case, however, (excepting plots of 6/242 of an acre and above in sub-block IV) is the plot variance found higher than division variance. Again, when the plot-size is between 12/242 and 16/242 of an acre there is a successful elimination of block variance in all the sub-blocks; giving to the size of that order a definite bias. Lastly, the need for a sufficient number of replications in a field experiment is brought out; thus a plot-size of 1/242 acre with forty-eight replications gives a higher standard of accuracy than a size of 2/242 acre with twenty-four replications. The size of 12/242 acre which we have considered to be suitable from other stand-points can be replicated only four times in each of the sub-blocks.

TABLE XVI

Plot size (Figures within brackets represent acreage)	Degrees of freedom		Total sum of squares		Variance		Whether difference in the previous columns is significant or not
	Divisions	Within divisions	Sum of squares due to divisions (Per cent of total)	Sum of squares due to within divisions (Per cent of total)	Due to divisions	Due to within divisions	
Sub-block I							
60′ × 3′ (1/242)	47	192	36	64	2,353	989	Significant
60′ × 6′ (2/242)	23	96	46	54	6,366	1,697	,,
120′ × 3′ (2/242)	23	96	43	57	6,672	2,070	,,
60′ × 9′ (3/242)	15	64	38	62	10,216	3,770	,,
240′ × 3′ (4/242)	11	48	35	65	11,952	5,041	,,
120′ × 6′ (4/242)	11	48	36	63	16,968	6,665	,,
60′ × 18′ (6/242)	7	32	45	55	38,255	10,181	,,
120′ × 9′ (6/242)	7	32	36	64	25,639	9,722	,,
480′ × 3′ (8/242)	5	24	40	60	36,135	10,962	,,
240′ × 6′ (8/242)	5	24	23	77	24,063	16,770	Not significant.
240′ × 9′ (12/242)	3	16	16	84	33,175	31,791	,,
120′ × 18′ (12/142)	3	16	42	58	1,25,775	32,256	Significant.
480′ × 6′ (16/242)	2	12	19	81	67,504	45,284	Not significant.
Sub-block II							
60′ × 3′ (1/242)	47	192	36	64	1,884	818	Significant.
60′ × 6′ (2/242)	23	96	39	61	4,379	1,616	,,
120′ × 3′ (2/242)	23	96	36	64	4,011	1,722	,,
60′ × 9′ (3/242)	15	64	35	65	5,905	2,611	,,
240′ × 3′ (4/242)	11	48	37	63	11,778	4,679	,,
120′ × 6′ (4/242)	11	48	40	60	11,797	4,084	,,
60′ × 18′ (6/242)	7	32	31	69	15,430	7,242	,,
120′ × 9′ (6/242)	7	32	25	75	12,583	8,172	Not significant.
480′ × 3′ (8/242)	5	24	32	68	27,266	12,254	Significant.
240′ × 6′ (8/242)	5	24	34	66	28,235	11,342	,,
240′ × 9′ (12/242)	3	16	33	67	36,751	13,619	,,
120′ × 18′ (12/242)	3	16	13	87	15,999	19,881	Not significant.
480′ × 6′ (16/242)	2	12	43	57	87,354	18,605	Significant.
Sub-block III							
60′ × 3′ (1/242)	47	192	28	72	1,593	992	Significant.
60′ × 6′ (2/242)	23	96	23	77	2,902	2,275	Not significant.
120′ × 3′ (2/242)	23	96	31	69	4,580	2,372	Significant.
60′ × 9′ (3/242)	15	64	26	74	6,231	4,007	,,
240′ × 3′ (4/242)	11	48	29	71	11,862	6,626	,,
120′ × 6′ (4/242)	11	48	22	78	8,195	6,464	Not significant
60′ × 18′ (6/242)	7	32	33	67	17,328	7,569	Significant.
120′ × 9′ (6/242)	7	32	26	74	18,860	11,642	Not significant.
480′ × 3′ (8/242)	5	24	34	66	48,104	17,030	Significant.
240′ × 6′ (8/242)	5	24	16	84	17,441	18,659	Not significant.
240′ × 9′ (12/242)	3	16	7	93	17,279	39,760	,,
120′ × 18′ (12/242)	3	16	37	63	69,108	21,756	Significant.
480 × 6′ (16/242)	2	12	13	87	59,634	61,157	Not significant.
Sub-block IV							
60′ × 3′ (1/242)	47	192	64	36	5,378	762	Significant.
60′ × 6′ (2/242)	23	96	62	38	17,173	2,460	,,
120′ × 3′ (2/242)	23	96	67	33	14,516	1,689	,,
60′ × 9′ (3/242)	15	64	57	43	32,926	5,837	,,
240′ × 3′ (4/232)	11	48	65	35	33,015	3,906	,,
120′ × 6′ (4/242)	11	48	44	56	45,885	15,178	,,
60′ × 18′ (6/242)	7	32	43	57	91,971	25,728	,,
120′ × 9′ (6/242)	7	32	61	39	1,00,255	13,877	,,
480′ × 3′ (8/242)	5	24	58	52	71,728	10,920	,,
240′ × 6′ (8/242)	5	24	71	29	1,27,626	10,486	,,
240′ × 9′ (12/242)	3	16	48	52	1,96,348	38,338	,,
120′ × 18′ (12/242)	3	16	39	61	2,59,229	75,680	,,
480′ × 6′ (16/242)	2	12	61	39	2,90,985	30,276	,,

Block variation

In all our calculations, we have tacitly assumed that each block contains five plots. The essential criterion for a suitable block size is that while it should be such that it should typify the major soil variation obtaining in the field as we pass from one point of the field to another and that the plot in each block should not be too small to lose its identity in the block in which it is placed. Table XVII gives the coefficient of variation for different division sizes in the several sub-blocks. In the first three sub-blocks, the block variability goes down till the division size is 60/242 or 80/242 of an acre, (*i.e.*, with the corresponding plot-size 12/242 or 16/242 of an acre) ; and in the fourth sub-block the variability is least when the division size is 80/242 of an acre.

But the block-size depends upon the number of treatments to be compared, and in the present case we have assumed five plots for each block. It is worth while, however, to consider the effect on the S. E. of an assumption of four, six and increasing number of plots in each block. We see, on the basis of five plots, that the block variation is small enough (as may be seen from Table XVII), and thus a comparison of five treatments (or approximately five) seems suitable under the soil conditions of the experimental field. It should be remembered, however, that the number of treatments to be compared cannot be unduly large, thereby increasing the block-size to an abnormal extent and diminishing the local control.

Table XVII

Coefficient of variation for different division sizes i.e., *in usual language block sizes* (i.e., $\frac{Block\ S.D.}{Block\ Mean} \times 100$)

Division size (acreage)	Sub-block I	Sub-block II	Sub-block III	Sub-block IV
5/242	2·8	2·6	2·4	4·9
10/242	2·3	1·9	1·6	4·4
	2·7	1·9	2·1	4·1
15/242	1·9	1·5	1·6	6·8
20/242	1·7	1·6	1·6	3·1
	1·9	1·6	1·4	3·6
30/242	1·9	1·2	1·3	3·4
	1·6	1·1	1·4	3·5
40/242	1·4	1·2	1·6	2·3
	1·1	1·2	1·0	2·9
60/242	0·9	0·9	0·7	3·5
	1·6	0·6	1·4	2·9
80/242	1·3	1·1	0·9	2·2

OTHER CONSIDERATIONS FOR FIXING A SUITABLE PLOT-SIZE

Every experimenter knows that in a field experiment with small plots, ' border effect ' is a very important consideration for drawing any valid inference. The statistical criteria for testing the border effect in the case of a field trial are, firstly whether the standard error of the experiment changes in any way by the exclusion of border rows,and secondly whether the comparative effects of different treatments with the whole plots harvested are in any way changed by the exclusion of border rows from the plot. We cannot however apply both the criteria in the case of a uniformity trial, but applying the first test for the first sub-block to the plot-sizes with a minimum of three rows in the case of the present data, it is found that the coefficient of variation has increased in every case by the exclusion of border rows.

The following table gives the results :—

TABLE XVIII (*a*)

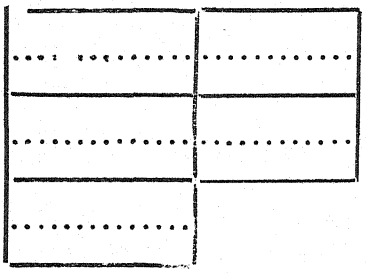
Plot-size	Coefficient of variation by harvesting the whole plot	Coefficient of variation by excluding two border rows
1 cut × 3 rows = 3/242 acre . .	6·0	9·3
1 cut × 6 rows = 6/242 acre . .	4·8	5·6
2 cuts × 3 rows = 6/242 acre . .	4·9	6·5
2 cuts × 6 rows = 12/242 acre . .	4·3	4·8
4 cuts × 3 rows = 12/242 acre . .	4·4	4·5

It is clear that in the case of the present trial, the exclusion of borders has produced a significant change in the coefficient of variation. This is in accordance with the results obtained by Immer [1932]. The question of competition has been further examined by working out correlation coefficients between the yields of any row and the mean of yields of adjacent rows in the first sub-block.

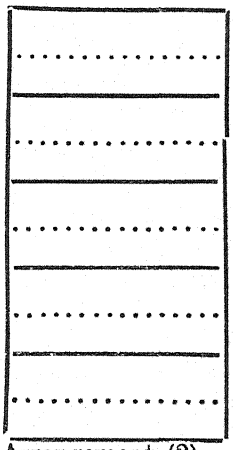
The value of correlation coefficient obtained was +0·1281 (not significant). This shows that the competition between rows is negligible in the case of the present data ; if there should be any competition, the correlation should be negative and significant, unless the soil differences concealed any competition that might be present.

Another important consideration connected with the lay-out of an experiment is whether the mode of arrangement of plots in a block exercises any influence on the standard error of the experiment. It is a common experience that more compact a block is, the less the standard error. For example, of the two arrangements

given below of the same plot-size the arrangement (1) will probably give a lower standard error than (2), as in the former case a greater proportion as ' block variance ' may be removed by analysis.



Arrangement (1)



Arrangement (2)

A fundamental problem in connection with the lay-out of an experiment is whether Latin square arrangement or randomised block method is suitable. While the latter has the advantage that any number of blocks or replications can be had, the former has the advantage, in leading to an effective elimination of variances in two directions. The types of experiment however, (*i.e.*, whether it is a varietal trial, manurial or irrigation) determines to a large extent the method of lay-out to be adopted.

From the present data of the uniformity trial, Table XVIII (*b*) has been calculated for a few cases only, to see which of the two methods of lay-out gives a low coefficient of variation. The figures generally show that except in cases of randomised block arrangement where an adequate number of replications is adopted (say five or six), the Latin square arrangement is found more efficient than the randomised block arrangement.

*The data further show that the decrease in the coefficient of variation with the increase in the number of replications (as the basis of the same number of plots in each block) is not consistent with theoretical expectations. Thus one would expect the coefficient of variation of five varieties with six replications to be $\sqrt{\frac{3}{6}}$ times the coefficient of variation of five varieties with three replications; in the present case it is much greater than what may be expected. This may be accounted for by the fact that the proportion of block variance eliminated in the analysis of variance is not always consistent with the number of replications adopted, but depends to a considerable extent upon the variation in the blocks themselves.

*Thanks are due to Mr. Yates, Chief Statistician, Rothamsted, for having brought this point to the notice of the authors.

TABLE XVIII (*b*)

Sub-block I

Average coefficient of variation for different types of lay-out

Plot-size	Latin square type			Randomised block arrangement			
	3 × 3	4 × 4	5 × 5	5 varieties × 3 replications	5 varieties × 4 replications	5 varieties × 5 replications	5 varieties × 6 replications
2 cuts × 3 rows = 6/242 acre	5·0	4·8	4·6	5·6	5·2	5·1	4·8
4 cuts × 3 rows = 12/242 acre	4·0	3·9		4·4	4·1		
8 cuts × 2 rows = 16/242 acre	3·7						

TABLE XIX

Theoretical number of replications to bring down the C. V. to two per cent

Length of cut (60 ft.)	Rows (between row to row 3 feet)			
	1	2	3	6
		Sub-blocks I—III		
1	20	10	8	5
2	11	8	6	4
4	7	5	4	
8	4	3		
		Sub-blocks I—IV		
1	20	12	10	8
2	12	12	7	6
4	7	5	5	
8	4	4		

THEORETICAL NUMBER OF REPLICATIONS NECESSARY TO BRING THE C. V. TO TWO PER CENT.

Assuming that the standard deviation per plot for different plot-sizes which we have deduced in Tables IV–VII are those which could be normally expected, the question is how many replications are necessary to bring the coefficient of variation to a low level say to two per cent.

The object of replications being to secure a low error, the number of replications necessary for each plot-size should be examined to see whether we could afford sufficient land consistent with the theoretical number of replications required.

Table XIX gives the theoretical number of replications necessary to bring down the coefficient of variation to two per cent on the basis of the data given in Table XI. For example, coefficient of variation for two rows and four cuts is 4·6 from Table XI; to bring it down to two per cent on this basis, the number of necessary replications is obtained from the formula : $\frac{4\cdot6}{\sqrt{n}}=2$, *i.e.* $\sqrt{n}=2\cdot3$ or $n=5\cdot3$.

Thus five replications of the particular plot-size will be necessaryto reduce the coefficient of variation to two per cent. With a coefficient of variation of two percent in a single mean, the coefficient of variation of the difference of two means is $2\sqrt{2}$ per cent and the difference in two means necessary for significance will be $2\sqrt{2} \times 2$, *i.e.*, 5·66 per cent ; and the number of replications given in Table XIX will be sufficient to bring down the error in the case of each plot-size to this level of significance. In the case of size of two cuts × six rows, (*i.e.*, 120 ft. × 18 ft. = 12/242 acre) the number of replications required will be four on this basis.

TABLE XX

Extent of land necessary for different plot-sizes to answer the theoretical number of replications (the nearest integer being taken in each case from Table XIX) and assumption of five plots in each block

Length of cut (60 ft.)	Rows			
	1	2	3	6
	Average of sub-blocks I—III (in $\frac{1}{242}$ acres)			
1	100	100	120	150
2	110	160	180	240
4	140	200	240	
8	160	240		
	Average of sub-blocks I—IV (in $\frac{1}{242}$ acres)			
1	100	120	100	240
2	120	240	210	360
3	140	200	300	
8	160	320		

EXTENT OF LAND NECESSARY BASED ON THEORETICAL NUMBER OF REPLICATIONS

Table XX shows the extent of land necessary for different plot-sizes on the basis of the theoretical number of replications given in Table XIX and on an assumption of five plots in each block. Even if the number of plots in each block changes, the ratios of the extents of area as shown in Table XX will remain the same. Taking the size 2 cuts × 6 rows, the number of plots in each block five, and the number of replications four (to bring down the C.V. to two per cent), the extent of land necessary is $12/242 \times 5 \times 4 = 240/242$ acre ; while for a size 2 cuts × 3 rows the extent of land necessary will be $6/242 \times 5 \times 6 = 180/242$ acre ; and so on. It is thus seen that on the basis of results of the first three sub-blocks, from the point of view of economy in land, of the one-cut plots, 1 cut × 1 row or 1 cut

× 2 rows is the most economical ; of two-cut plots 2 cuts × 1 row is most economical and so on. But we have already seen that from other considerations six-row plots are suitable and that more than one cut to bring down the S. E. a plot-size of 6 rows × 2 cuts, (*i.e.*, 12/242 acre) demands an extent of 240/242 acre seems suitable. But costs of cultivation, planting and harvesting are not always proportional to the areas cultivated in view of some constan t expenditure involved in cultivation. It is therefore necessary that in case when between two or three plot-sizes one has to be decided upon not only is the extent of land the main consideration but also costs of cultivation involved.

Comparison of work in other countries with different crops

Though the size of the experimental plot for severa l crops under different conditions has been engaging the agricultural experim enters in other countries, so far no special attention has been paid to the shape of the plot. The question of shape has been studied by Christidis [1931] and his conclusions are :—

(1) In agricultural experiments it seems that significant results cannot be secured by only using appropriate statistical methods ; uniformity amongst the individual plots is more essential than anything else.

(2) In order to test the validity of the assumptions m ade regarding the effect of the shape of the plots, the numerical data of several uniformity trials have been considered (on mangolds, potatoes, oats, and green forage). A close agreement was found between expectation and actual results in the great majority of cases, the evidence being remarkably significant in favour of the long plots. In only three cases were the results inconclusive, this apparently being accounted for by the way in which the original plots were formed, causing an inequality in area amongst them.

(3) In the light of these investigations, in order to reduce the effect of soil heterogeneity, the plots used should be as long and narrow as possible. This, of course within the limits set by different practical considerations, amongst which convenience, competition (when acting), and the accurate measurement of the width appear to be the most important.

These conclusions tally remarkably with our own inferences on the data of the present experiments on un-irrigated sugarcane.

Summary and conclusions

1. The need for standardising a suitable size and shape for an experimental plot for all crops in different farms in India grown under different conditions is very urgent ; this question is assuming a definite importance in the case of sugarcane which is now one of the most important economic crops in India. It is further important to recognise the need for a correct statistical technique to be applied to data from experiments conducted for the purpose of deducing a suitable size and shape for an experimental plot.

2. An experiment was conducted on unirrigated sugarcane (Co. 205) in Harpur Jhilli in 1932, with a view to deduce a suitable size and shape for the experimental plot. The yield figures for 840 plots of plot-size 30 ft.-3 in. × 3 ft. were available. The coefficient of variation was worked out for each plot-size and though broad conclusions were possible from these data, it was found that a high variation in fertility between plot and plot and row and row was a disturbing factor, and it was therefore thought advisable to repeat the experiment in 1933 on a more popular variety of cane than Co. 205 and on a soil of more uniform fertility.

3. The 1933 experiment was conducted at Meghaul (Monghyr District) on Co. 213 cane. This variety was bred by Dr. C. A. Barber at Coimbatore. Its high value in the mill trial, its remarkable tonnage capacity, and its agricultural habit on an estate scale were first tested by the Secretary, Sugar Bureau, at present the Imperial Agriculturist, Pusa. It now occupies the first place as regards area under cultivation in Bihar, Bengal and the United Provinces. Its average tonnage in the Pusa Farm is twenty-two tons per acre.

4. The land under the present experiment was under regular sugarcane rotation in previous years ; cultural methods, manuring and other operations were uniform all over the area. The lay-out of the experiment consisted of, after removing the border crop, two blocks of sizes 480 ft. × 315 ft. and 480 ft. × 93 ft. In the first block there were 105 rows and in the second 31 rows—the rows being separated by three feet. Each row was cut in eight equal sections and the ultimate plot thus measured 60 ft. × 3 ft. = 1/242 acre. The field was divided into four sub-blocks, each measuirng nearly one acre, for the purpose of this study (which necessitated the omission of a few rows).

5. Analysis of variance in yields has been worked out for : (*a*) blocks and (*b*) within blocks for the first three sub-blocks and also for all the four sub-blocks. Coefficient of variation V and its standard error, have been calculated for each plot-size in the several sub-blocks.

6. It is found that the variation in fertility between the first three sub-blocks is not marked, and that the fourth sub-block is found definitely inferior in fertility to the other sub-blocks.

7. All the values of coefficient of variation are found to be high as compared to their standard error, showing their reliability.

8. Based upon trend in the values of coefficient of variation a plot-size between 12/242 acre and 16/242 acre seems desirable.

9. The shape of the plot has to be decided not only on the basis of trend in the coefficient of variation, but also from the point of view of convenience of cultivation, and an irreducible minimum number of rows to avoid border effect ; based upon these standpoints the ratio of length to width of the experimental plot is found to be at least 6·7. Long narrow plots are thus found to sample the soil best,

and this tallies with the conclusions reached by Christidis, published in *Journal of Agricultural Science*, January 1931, (pages 14—37).

10. From the point of view of efficiency, based upon standard error relative to size, the smallest plot-size is the most efficient, but there are other considerations, such as convenience in cultivation and reduction of border effect, that decide a suitable plot-size. In the present experiment, it is seen that between plots of the same shape, efficiency has not appreciably changed provided the plot is sufficiently narrow. The present data also emphasise the fact that between two plot-sizes one of which is to be preferred, the smaller size with a greater number of replications is more efficient than the larger size with a lesser number of replications.

11. Analysing the variances due to ' divisions ' and ' within divisions ' in the case of each plot-size, two points are made clear :—(1) The block demarcation in a field trial is essential and (2) in the case of the present experiment, a plot-size between 12/242 of an acre and 16/242 of an acre eliminates an appreciable portion of soil variance.

12. Examining other considerations for fixing a suitable plot-size, in the case of the present data the border effect is found significant but competition between rows is negligible.

13. Again it is found that between ' Latin square ' and ' randomised block arrangement ' the former is found more efficient than the latter, except when the latter is provided with a sufficient number of replications, say five or six.

14. Theoretical number of replications to bring down the coefficient of variation to two per cent has been calculated in the case of each plot-size, and it is found that in the case of 2 cuts × 6 rows (*i.e.*, 12/242 acre), the number of replications required is four.

15. Extent of land necessary on the basis of theoretical number of replications, and on the basis of five plots in each block, has been calculated in the case of each plot-size. It is found that a plot-size of 12/242 acre requires 240/242 of an acre for the whole experiment. Costs of cultivation are also an important consideration in the case of a field experiment, which should be worked out for different plot-sizes.

16. Considered from all stand-points on the basis of the data in the present experiment, a plot-size of 12/242 of an acre (120 ft. × 18 ft.) seems eminently suitable for a field experiment on un-irrigated sugarcane.

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Appendix

TONNAGE EXPERIMENT WITH Co. 213, 1933

Each row is 3 ft. apart and the cuts are 60 ft. Area of each section is 180 sq. feet

(Yield in pounds)

Row No.	A	B	C	D	E	F	G	H
				Block I				
1	389·0	382.0	360·0	306·5	264·0	321·0	354·0	360·5
2	302·0	369·0	415·5	320·0	293·0	293·0	344·0	376·5
3	261·0	328·0	306·0	239·5	305·0	295·5	303·5	334·0
4	337·0	310·0	319·5	367·5	288·0	344·0	313·5	330·0
5	314·5	341·0	342·5	322·0	265·0	331·5	416·0	335·0
6	356·0	371·0	345·0	280·0	242·5	350·5	342·5	389·0
7	384·0	353·5	368·0	303·0	322·0	363·5	372·0	363·5
8	330·0	377·5	402·0	445·5	313·5	366·5	360·5	334·5
9	338·0	362·5	340·0	356·0	376·0	380·0	392·0	407·0
10	311·5	346·0	345·5	343·5	334·5	339·0	393·5	396·5
11	346·5	335·0	389·0	321·5	357·0	353·5	333·0	315·5
12	323·0	402·5	324·5	372·0	372·5	334·0	327·0	305·5
13	351·5	314·0	375·0	420·0	392·0	374·5	337·0	308·5
14	402·5	333·5	351·0	405·5	327·5	311·5	321·5	332·0
15	346·0	364·0	377·0	333·0	326·0	366·5	392·0	339·5
16	393·5	303·5	363·0	326·0	338·5	352·5	413·0	413·0
17	320·0	387·0	402·5	342·5	320·5	299·0	306·0	357·0
18	328·0	337·5	387·5	305·0	311·0	360·5	389·5	359·5
19	336·0	368·0	302·0	400·0	325·0	354·5	341·5	376·0
20	327·0	335·0	365·0	332·0	270·0	269·0	338·0	350·0
21	352·5	322·5	344·0	330·5	242·0	361·0	371·5	355·5
22	358·0	352·5	325·0	349·0	315·0	350·0	389·0	370·0
23	357·0	330·0	343·5	319·5	315·0	305·0	347·0	355·0
24	337·5	366·5	371·0	299·5	310·5	305·0	323·5	359·0
25	312·5	339·5	348·0	324·0	309·0	283·0	339·0	308·0
26	362·0	306·0	338·0	331·0	259·5	358·5	355·5	340·0
27	290·0	412·0	357·5	305·5	312·0	367·0	334·0	365·0
28	318·0	363·5	349·5	354·5	257·0	289·0	314·0	347·0
29	315·5	353·0	398·5	348·5	286·0	350·0	332·0	290·0
30	325·0	337·0	359·0	289·0	287·5	316·5	339·0	345·0
31	354·0	360·0	387·0	338·0	350·5	281·0	368·0	348·0
32	340·0	330·5	370·0	342·0	321·5	314·5	339·5	370·0
33	355·0	370·0	393·5	319·5	317·5	290·5	348·5	330·0
34	373·0	393·0	333·5	376·0	328·5	335·5	385·0	355·0
35	314·0	353·0	388·5	378·5	364·0	310·0	331·5	330·0
36	341·0	351·0	385·0	358·0	293·0	369·5	327·5	341·5
37	295·0	362·0	387·0	363·0	315·0	319·5	320·0	322·0
38	328·0	355·0	351·0	358·0	343·5	334·0	327·5	338·0
39	295·0	324·0	364·5	351·0	322·0	316·0	336·0	305·0
40	307·0	364·0	379·0	323·0	316·0	345·0	331·0	335·0
41	339·0	340·0	301·5	326·5	394·5	361·5	402·0	369·5

Appendix—*contd.*

Row No.	A	B	C	D	E	F	G	H
42	365·0	360·0	275·5	351·0	357·0	363·0	328·0	380·0
43	360·0	363·0	348·5	357·0	336·0	366·0	279·5	352·0
44	372·0	346·5	360·0	343·0	388·5	360·5	407·0	327·0
45	304·0	369·0	261·0	360·0	359·0	260·0	295·5	382·0
46	332·0	289·0	312·5	289·5	346·0	362·0	317·0	380·0
47	317·0	285·0	348·5	343·5	372·0	304·5	321·0	347·5
48	380·0	305·0	341·5	381·0	352·0	339·0	410·0	380·0
49	366·5	350·5	332·5	365·0	330·0	326·0	350·0	347·5
50	357·5	340·0	299·0	350·0	350·0	336·0	316·0	330·5
51	325·0	338·5	349·5	361·5	319·0	343·0	318·0	385·0
52	391·0	324·0	311·5	331·0	322·5	298·0	250·0	357·0
53	313·5	315·0	286·5	331·0	350·0	311·5	272·0	333·5
54	337·0	308.0	264·0	385·0	341·5	322·5	334·0	299·0
55	326·0	305.0	301·0	310·0	242·0	305·5	321·5	312·5
56	393·0	319·5	311·5	361·0	308·0	359·0	342·5	365·5
57	395·0	294·5	359·0	342·0	339·0	354·0	410·0	378·5
58	230·0	288·0	321·0	284·0	313·5	377·5	293·0	333·5
59	363·0	330·0	371·0	298·5	304·5	323·0	374·0	354·0
60	375·0	317·0	333·0	306·0	309·5	305·0	330·5	382·5
61	347·5	358·0	356·0	307·0	295·0	282·0	283·0	342·0
62	312·0	271·0	318·0	305·0	313·0	254·0	325·0	311·0
63	277·0	327·5	326·5	330·0	158·0	267·0	295·0	320·0
64	275·5	300·0	340·0	364·0	427·0	308·0	365·0	371·0
65	378·0	345·0	340·5	364·0	315·0	322·0	342·0	347·0
66	315·0	280·0	346·0	322·0	361·0	335·0	316·0	335·0
67	329·0	327·0	315·0	347·0	354·5	378·0	349·0	350·5
68	333·0	340·0	313·5	301·5	337·0	350·0	338·0	338·5
69	356·0	323·0	413·0	422·0	353·5	368·0	342·0	314·5
70	333·0	303·0	328·5	376·0	315·0	336·0	359·0	384·5
71	321·5	337·0	339·5	297·0	335·0	377·5	356·0	349·0
72	291·5	310·0	345·0	295·0	339·0	331·0	344·0	311·5
73	315·5	320·5	340·0	333·5	337·0	357·5	301·0	365·0
74	348·5	300·0	332·5	345·0	327·5	315·5	340·0	345·0
75	318·0	286·0	292·5	317·5	270·0	307·5	317·5	318·5
76	269·5	327·0	285·0	316·5	324·5	286·0	355·0	315·0
77	276·0	365·0	388·0	308·0	307·0	348·0	362·0	356·5
78	316·5	378·0	310·0	345·0	305·0	308·5	318·5	333·5
79	337·5	362·0	334·0	342·0	323·0	305·0	350·0	372·0
80	266·5	366·0	322·5	330·5	267·0	335·0	335·0	309·5
81	312·0	374·0	361·5	425·0	383·0	340·0	370·0	425·0
82	319·0	403·5	334·0	335·5	326·0	367·0	401·0	367·0
83	400·0	308·5	284·5	357·5	355·0	354·0	320·0	341·0
84	328·5	270·0	315·5	341·0	375·0	340·0	370·0	356·0
85	325·0	288·5	332·0	374·0	325·5	349·5	338·0	295·0
86	321·0	325·0	312·0	343·0	300·0	345·0	306·5	326·0
87	315·0	280·0	305·0	308·0	343·0	333·0	332·5	316·0
88	278·0	333·0	338·0	332·0	386·0	322·0	340·0	317·5
89	309·0	286·0	341·0	328·5	385·0	369·5	370·0	313·5
90	326·0	325·0	343·5	339·0	362·0	327·5	321·5	316·5
91	314·5	288·0	330·0	306·0	356·5	331·0	305·0	272·5

Appendix—*contd.*

Row No.	A	B	C	D	E	F	G	H
92	320·0	343·5	340·0	286·0	286·0	274·5	290·0	285·0
93	336·0	315·5	313·0	313·5	328·0	295·0	320·0	312·5
94	333·0	315·0	258·0	308·0	296·5	310·0	315·5	327·5
95	345·0	347·0	342·0	328·0	359·0	312·0	332·0	321·0
96	354·0	303·0	287·5	269·0	361·0	323·5	342·0	360·0
97	345·0	262·5	293·0	322·5	325·0	314·0	370·5	356·5
98	322·5	272·0	298·5	356·0	327·0	373·0	375·5	351·0
99	293·0	261·0	206·0	295·0	271·5	330·0	358·0	343·0
100	313·5	289·5	274·5	336·5	368·5	331·5	333·5	294·5
101	291·5	265·0	242·5	321·0	317·5	367·5	333·5	301·0
102	331·0	271·0	279·0	298·5	342·0	313·0	371·5	283·5
103	351·5	290·0	309·5	340·5	273·5	315·0	310·0	329·5
104	315·0	266·5	335·0	315·5	310·0	340·0	319·5	324·5
105	276·5	232·5	285·0	285·0	330·0	316·0	352·0	320·0
				Block III				
106	276·0	285·0	279·5	319·5	397·5	341·5	324·0	253·0
107	280·0	252·0	243·0	328·5	395·0	330·0	245·0	275·0
108	297·5	268·5	303·0	280·5	373·5	348·5	284·5	278·0
109	279·5	285·0	324·0	343·0	357·5	330·0	290·0	293·0
110	332·5	285·0	325·0	333·5	372·0	303·5	258·5	290·0
111	212·5	242·0	315·0	255·0	318·5	287·0	268·0	336·0
112	356·0	340·0	335·0	277·5	318·0	356·0	311·5	306·5
113	330·0	292·0	314·5	342·5	296·0	350·5	300·0	335·5
114	315·0	277·0	259·5	300·0	327·5	346·5	276·0	362·0
115	300·0	280·0	310·0	288·0	331·0	411·0	307·5	332·0
116	263·0	256·5	294·0	319·0	335·5	391·0	265·5	392·5
117	310·5	228·0	215·0	308·0	322·5	335·0	304·5	311·0
118	343·5	214·0	280·0	328·5	302·0	317·0	305·0	345·5
119	256·0	236·5	260·0	294·0	337·5	342·0	306·5	297·0
120	328·0	257·0	292·5	367·5	383·0	379·0	295·5	343·5
121	270·0	216·0	306·5	325·0	323·0	328·0	304·5	306·5
122	266·5	199·0	262·0	340·5	330·0	321·0	330·0	312·0
123	231·0	207·0	294·5	343·0	312·0	339·0	262·5	229·0
124	287·5	234·5	258·5	347·0	338·0	350·0	280·0	250·0
125	294·0	261·5	295·5	277·0	312·0	329·5	300·5	267·0
126	262·5	303·0	293·0	304·5	323·5	370·0	273·5	250·0
127	307·0	279·5	304·5	285·5	310·0	350·0	255·5	288·5
128	264·5	253·5	233·0	290·0	284·0	285·0	302·0	258·5
129	319·5	262·0	259·5	306·0	264·0	315·0	316·0	264·5
130	315·0	245·5	271·0	269·0	251·0	263·5	297·5	276·0
131	280·0	236·5	293·0	250·0	228·5	274·0	291·0	295·5
132	313·5	265·0	266·0	300·0	250·0	287·5	310·0	303·0
133	291·5	197·0	230·0	298·0	225·5	280·0	233·0	374·0
134	293·5	229·0	226·0	277·0	232·0	290·0	261·0	307·5
135	339·5	225·0	216·0	304·0	220·0	297·5	303·0	290·0
136	248·5	210·0	205·5	281·0	290·0	301·0	239·5	285·0

THE BASIS FOR MERGING *SCLEROSPORA INDICA* WITH *SCL. PHILIPPINENSIS*

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(With Plate XLII)

Further study of the genus Sclerospora reveals the necessity for revising the status of certain species. In a previous paper Weston and Uppal [1932] presented the basis for establishing Kulkarni's variety *Andropogonis sorghi* of *Sclerospora granminicola* as a distinct species *Sclerospora sorghi*. In the present paper the status of *Sclerospora indica* Butler and of *Sclerospora philippinensis* Weston is considered, since recent comparative studies have revealed that the differences between these two are not sufficient to justify maintaining them as separate species.

Sclerospora indica, when first reported on maize from Pusa by Butler [1913], was regarded by him as identical with the maize mildew described as *Peronospora maydis* by Raciborski [1897] from Java in 1897. Butler, however, pointed out that the fungus was clearly a *Sclerospora* rather than *Peronospora* and hence designated it as *Sclerospora maydis* (Rac.) Butler. Later work by Palm [1918] corroborated Butler's conclusion that the Javan mildew was indeed a *Sclerospora* but showed that it was specifically distinct from the Indian mildew ; hence in 1931 Butler and Bisby [1931] very properly re-named the latter *Sclerospora indica*.

Sclerospora philippinensis, when described from the Philippines as a new species by Weston [1920] in 1920, had already previously been reported from the Philippines in 1916 by Baker [1916], who first called attention to its destructiveness to maize, and in 1918 by Reinking [1918], who first described and illustrated the structural characters of the fungus.

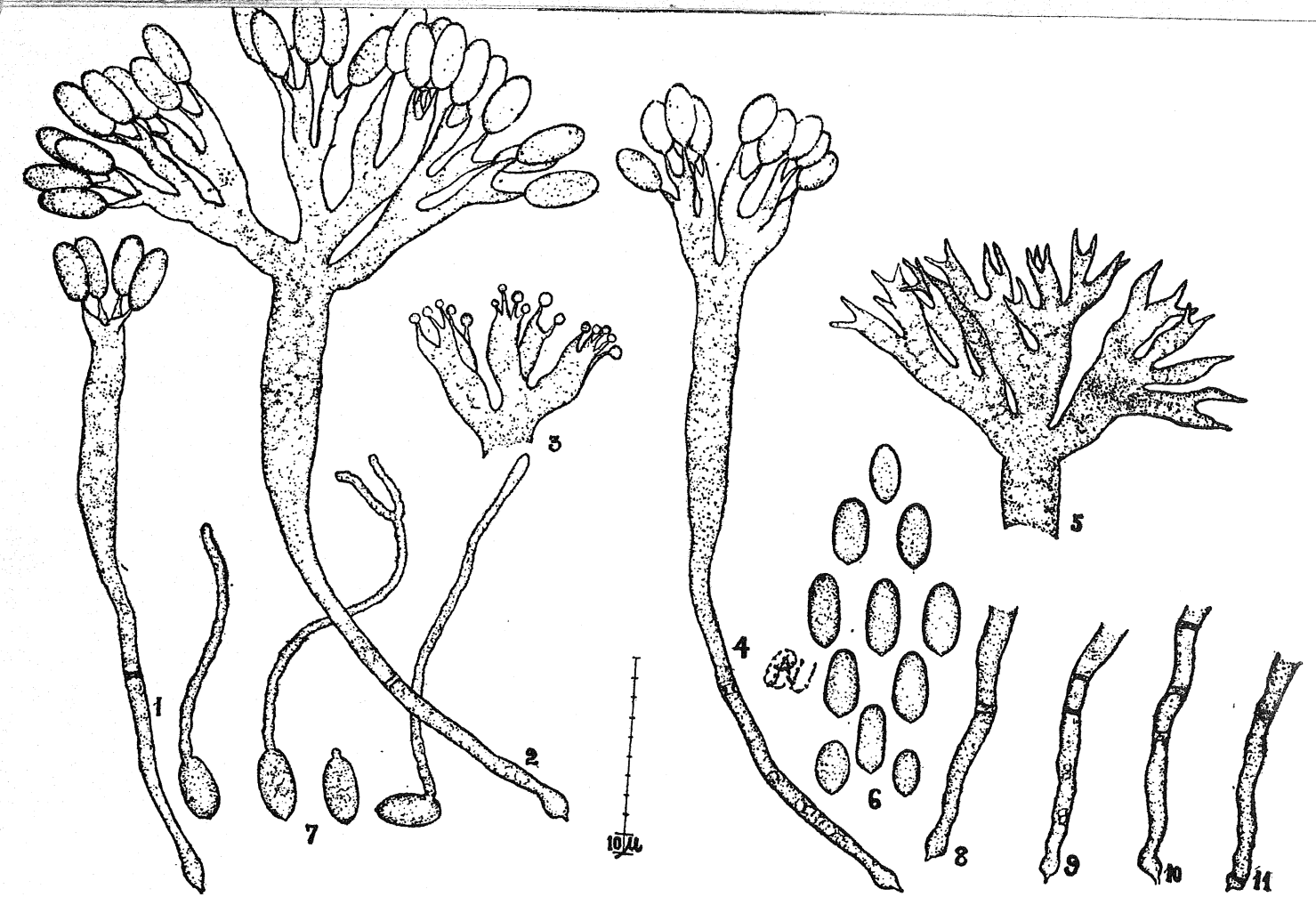
Both Baker and Reinking assumed that the Philippine fungus was identical with that reported from India by Butler, the general impression at the time being that the destructive downy mildew of maize in the Orient was a single entity first reported from Java, later found in India, then in the Philippines and probably already established and awaiting recognition in other countries as well.

In 1920, however, when Weston was confronted with the question of the identity of the Philippine maize mildew, the situation had become more complex. Palm had shown that the species in Java was distinct from that in India, whilst in Australia D. S. North had encountered a similar, although distinct, mildew destructive to sugarcane and had called the attention of Lyon [1915] to Miyake's little known report [1911] describing this species as *Scl. sacchari* and noting its destructiveness to maize as well as sugarcane in Formosa. Moreover Weston, during his investigation of the Philippine mildew, having found that the production of conidiophores and conidia in the field normally occurred at night when the host plants were covered with dew, had worked out the structural characters in detail and from comparisons with dried herbarium material, had concluded that an adequate concept of the distinguishing diagnostic features could be gained only from material collected at the time of optimum production and studied either when fresh or when suitably killed and preserved. At that time, the only material of the Javan, Indian and Formosan species available for comparison consisted of inadequate dried herbarium specimens, which even when supplemented by the published descriptions hardly gave adequate basis for decision even though indicating that the Philippine fungus was apparently distinct from the Javan and Formosan species, and probably from the Indian species as well.

Confronted with these difficulties Weston [1920] concluded that the best way to meet the situation was to regard the Philippine fungus as a separate species and emphasised (p. 119) " the need of comparative studies using optimum material " to settle the question of identity conclusively.

In recent years the writers have been given the opportunity of making such a comparison of *Sclerospora philippinensis* with *Scl. indica*. Uppal [1930, 1931, 1932], in the course of his investigations into various *Sclerosporas* at Poona, has studied carefully preserved material of *Scl. indica* from Pusa kindly furnished to him by Dr. W. McRae, and has been increasingly impressed by its agreement with *Scl. philippinensis*. The living material * of the maize *Sclerospora*, collected at the time of optimum production in the field at Pusa, has also been studied. Recently Weston has compared excellently preserved material of the Indian fungus from Pusa with his own Philippine material already described [Weston, 1921, 1923] and has corroborated Uppal's conclusion as to their agreement.

* Mr. M. K. Desai assisted the writers in making measurements of the living material of *Sclerospora indica* collected under conditions of optimum production at Pusa, Bihar.



1. A slender conidiophore which has failed to branch and has terminated in conidia directly. 2. A well-developed, mature conidiophore with conidia. 3. Portion of a conidiophore showing conidia just budding out. 4. A slender, sparingly branched conidiophore bearing few conidia. It is partially mature as can be seen from the rotund shape of conidia and from the incomplete development of the basal septum. 5. Portion of a profusely branched conidiophore after the conidia have been detached from the sterigmata. 6. Conidia showing the range of variation in shape and size. 7. Conidia showing direct germination by hyphae. 8, 9 and 10. Bases of conidiophores showing one, two and three septa, respectively. 11. Base of a conidiophore showing two septa, one of which is located at the extreme basal tip, a condition not normally encountered.

(Magnified 275 times).

PLATE XLII

These comparative studies of the optimum material conclusively show that the slight differences between the Indian and the Philippine maize mildews do not justify maintaining these two as separate species. The agreement between these two fungi holds in all their essential features.

The conidiophores are, of course, of the well-developed type characteristic in general of the conidial *Sclerosporas* of the Orient with a differentiated basal cell (Plate XLII, figs. 1, 4, 8-11), stout main axis (Plate XLII, figs. 1, 2, 4), and extensive spreading branch system (Plate XLII, figs. 2-5), comprising stout, usually dichotomous branches terminating in the tapering sterigmata which bear the spores.

These features are well shown in Plate XLII included here to supplement Butler's [1913] original illustrations which unfortunately were based on incomplete specimens poorly developed and lacking the characteristic basal cell. A comparison of the present figures with those of Weston's [1920, 24-25] earlier illustrations of the Philippine material shows close agreement in all essential structural features. Furthermore, the dimensions are in close agreement : the total length of the conidiophores in both ranges from 150 to 410μ, most commonly from 300 to 350μ, the greatest diameter of the main axis in both is most commonly from 20 to 25μ, and the basal cell is typically about 80 to 100μ in length, and 9 to 11μ in diameter. Even the sterigmata, which were emphasised by Weston [1920, p. 117] as a point of difference, are now found to be more closely in agreement, since they vary greatly in length from as little as 6μ to as much as 30μ but average around 14 to 15μ.

The conidia, which have been found to be the most reliable basis of comparison in the species of *Sclerospora*, here show close agreement both in shape and in size. In shape they are most commonly elongate ellipsoid, elongate ovoid, or rotund cylindric (Plate XLII, fig. 6 and Weston's [1920] Plates 24 and 25). In size, although there are slight variations in the modes of length and of width, there is nevertheless a distinct overlapping in the length frequencies, sixty per cent of the Indian material falling in the range of 33 to 41μ, and sixty-five per cent of the Philippine material in the range of 31 to 39μ. In width, however, the frequencies overlap over almost the entire range of spore measurements, although the conidia of *Scl. indica* are slightly narrower (Table I). As has been suggested by Dr. Butler in a recent letter, these slight variations in conidial measurements may be regarded as characteristic of a geographical race ; for it has been his experience in comparing different samples of material received at the Imperial Mycological Institute from collections in widely separated localities that such material may show substantial differences in spore measurements even though belonging to the same species of fungus causing the same disease in the same host. Such differences in size, therefore, unless very pronounced, with no intermediates and augmented with additional structural distinctions, do not seem to justify recognition of the two mildews as separa te species.

TABLE I

Comparison of measurements of conidia of Scl. indica *and* Scl. philippinensis

Length			Width		
Classes in μ	Number of conidia in 400		Classes in μ	Number of conidia in 400	
	Scl. indica	** *Scl. philippinensis*		*Scl. indica*	*Scl. philippinensis*
17 to 19	0	1	11 to 13	5	1
19 to 21	0	1	13 to 15	40	8
21 to 23	3	2	15 to 17	135	41
23 to 25	3	1	17 to 19	97	160
25 to 27	3	4	19 to 21	53	148
27 to 29	4	10	21 to 23	51	41
29 to 31	11	35	23 to 25	14	1
31 to 33	24	68	25 to 27	5	0
33 to 35	42	75			
35 to 37	46	64			
37 to 39	79	55			
39 to 41	75	30			
41 to 43	36	24			
43 to 45	31	21			
45 to 47	23	7			
47 to 49	9	2			
49 to 51	5	0			
51 to 53	3	1			
53 to 55	1	0			
55 to 57	2	0			

* Conidial material collected at the time of optimum production (4 a.m.) at Pusa and measured immediately by M. K. Desai.

** Conidia collected by Weston in the Philippines in dew during optimum conidium production and measured immediately as reported in *J. Agric. Res.* **19**, No. 3, 1920 (Table I).

As a result, when all these points are considered there does not seem to be any adequate basis for maintaining *Sclerospora philippinensis* Weston of the Philippines and *Sclerospora indica* Butler found only in Bihar in India, as separate species. It is most regrettable that this is the case, for this *Sclerospora* should, in all justice, give recognition, in the authority for its name, to Butler who first called attention to it in 1913. Yet the early confusion in identity with the Javan fungus and the unfortunate taxonomic tangle which followed Palm's failure to observe the International Rules of Nomenclature in re-naming Raciborski's *Peronospora maydis* forced Butler to re-name his species *Sclerospora indica* in 1931. As a result now that our comparative study reveals no adequate basis for maintaining *Sclerospora indica* and *Scl. philippinensis* as separate species, the former must be merged with the latter under the name *Sclerospora philippinensis* Weston.

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THE TOLERANCE OF WHEAT PLANTS FOR CHLORIDES OF CERTAIN NON-ESSENTIAL ELEMENTS*

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(With eight text-figures)

INTRODUCTION

The growing interest in the problems of plant nutrition within recent years has resulted to a certain extent in the realisation of the possible relation existing between the nature and amounts of elements absorbed and the elaboration of the several organic substances at different stages of growth. Such experiments conducted under natural and controlled conditions of experimentation have led to the view that growth is mainly dependent upon a number of the so-called essential and non-essential elements. While the former are required in definite quantitative proportion, the presence of the latter in minute quantities is considered to be necessary for the proper development of plants.

The investigations of Mazé [1914 ; 1915 ; 1919], McHargue [1919 ; 1922 ; 1925 ; 1927], Warington [1923 ; 1926], Brenchley [1914 ; 1, 2], Brenchley and Thornton [1925], Sommer and Lipman [1926], and Haas and Reed [1927], besides a host of other workers, have shown how the growth of their experimental plants varied in magnitude according to the presence or absence of some of these non-essential elements and in certain cases stress has been laid on the indispensability of these elements to the growth of plants. Such a relation between growth and availability of these non-essential elements, however, remains not well understood specially with respect to the tropical and sub-tropical crops and other characteristics of these regions. The need for a systematic investigation in this direction is the justification for the present contribution.

*(Contribution from the Institute of Agricultural Research, Benares Hindu University.)

To this end, wheat plants are raised in Knop's solution under varying concentrations of the supply of these elements and the subsequent variations in certain aspects of the morphology, growth and composition of the plants are studied with respect to shoot and root lengths, leaf area, dry matter formation and the final elaboration of certain organic substances such as the various carbohydrates as well as the organic and inorganic nitrogen. To further facilitate the analysis of the relative efficiency of the many non-essential elements under investigation their chlorides are added to the culture solution in suitable molar concentrations. The experiments are performed under rigidly controlled conditions of other conditioning variables, such that limitations in these directions may not interfere in the deduction of any genuine conclusion with respect to the influence of these elements on plant growth.

Investigation

Pure strain seeds of *Triticum vulgare* (*var.* Pusa 4) of as uniform a weight and size as possible are selected, sterilised with mercuric chloride (1 : 1,000) and then washed well with distilled water. They are then germinated on mosquito nettings moistened with Knop's solution, under controlled environmental conditions. When the seedlings have grown to the extent of approximately 6 cm., they are further selected with regard to their uniformity in size and vigour and are then transferred to wide-mouth culture jars of hard resistant glass, 500 c.c. in capacity. The glass containers are covered with black paper and are fitted with perforated corks thoroughly impregnated with molten wax. The vessels together with corks are sterilised before the addition of the culture solution and only two plants are allowed to grow therein.

Three series of experiments have been conducted. In the first, the plants are grown exclusively in Knop's solution throughout their life-cycle and designated as the control, while in the other two, seedlings raised in Knop's solution for a week, are transferred to this very solution containing the chlorides of cobalt, iodine, zinc, aluminium, manganese, lithium and sodium. Four concentrations of each chloride, namely 0·01, 0·005, 0·0005 and 0·0001*M*, are tried, out of which the first two belong to the second series and the last two, to the third. Such a wide range of concentrations has been specially utilised to study the effect of the different elements when supplied both in strong and weak doses and thereby to discover the optimal range of concentrations which may lead to useful stimulating results.

The plants are grown in the water culture laboratory where other environmental factors are kept under rigid control. The nutrient solution is changed twice a week and aerated every day. A free circulation of air is maintained in the laboratory in order to supply fresh oxygen and carbon dioxide to the plants throughout the entire series of experimentation.

At intervals of a week, a number of plants are taken out from each set and washed free of nutrients. The external moisture is carefully wiped off before the fresh weight of the entire plant, as well as such measurements as root and shoot

lengths, leaf area and other morphological characteristics, are recorded. The plant material is then fixed in five per cent formaldehyde, washed well with distilled water and then incubated to constant dry weight in a steam oven regulated at 100°C.

The materials for the estimation of the various carbohydrates (glucose, sucrose and starch including the petosans and other acid-soluble carbohydrates) are prepared after the method of Link and Tottingham [1923] and then estimated according to Official and Tentative Methods of Analysis [1930]. The total nitrogen including the nitrogen of nitrates is obtained by the modified Kjeldahl's method [1930].

Experimental findings

Morphological variabilities.—The morphological variations attendant upon the addition of different chlorides in varying concentrations to the culture solution range from a stage of moderate acceleration to that of complete toxicity. In case of certain chlorides in the second series, both the concentrations (0·01 and 0·005*M* solutions) result in an immediate injury, whereas in others, the higher concentration (0·01*M*) is severely toxic to the plants. The injury caused by the application of the lower concentration (0·005*M*) is, however, mild and not so well pronounced as in the higher one. To the first group belongs the most characteristic case of the cobalt chloride, the toxic nature of which in 0·01*M* concentration is evinced soon after thirty-six hours of its addition. The leaves change their normal colour from green to bluish dark and later to greenish purple. The normal turgidity is lost and the leaves wilt off badly. In four or five days the entire plant population is severely affected culminating in their death. The lower concentration, although producing more or less similar morbid symptoms, is not so toxic as to cause immediate injury. The plants so treated survive longer for a period of a week or so.

In case of iodine chloride, on the other hand, similar toxicity is evinced in both the concentrations, but the injury is manifest after a few days of the growth of plants in this solution. The roots assume a reddish tinge and cease to grow, the leaves wilt and droop down and ultimately the entire plant is killed. In the case of higher concentration (0·01*M*) these symptoms appear as early as four days after the transfer and the plants do not survive for more than a week.

Zinc chloride, on the other hand, takes a still longer time to manifest its toxic effect which is more pronounced in case of higher concentration (0·01*M*). About a week after the plants have been transferred to this solution, a few leaves begin to show signs of chlorosis and by the end of another week the foliage almost dries up, while the leaves that are left, remain yellow and completely chlorotic. Thus, two weeks later, in case of the higher concentration and three weeks in the lower one, almost all the plants are killed by the toxic nature of the salt—which is well in accord with the observations of Conner [1920].

It is thus seen that the plants grown in culture solution with different chlorides of 0·01 or 0·005*M* strength are soon affected by their toxic action finally culminating in their death. There are yet other chlorides which, though detrimental for plant growth, allow the plants to continue their growth and complete their life-cycle when added to culture solution in 0·005*M* strength. This has been shown to be specially the case with aluminium chloride. In this particular case, although most of the plants are killed four weeks after the transfer in 0·01*M* concentration, some of the plants in 0·005*M* solution do survive the toxic effect and ear earliest of all the cultures of the second series. The toxic nature of this salt too, however, is manifest soon after its addition to the nutrient media, by the cessation of root development followed by the loss of its normal colour. Afterwards the roots get slimy to touch and gradually begin to disintegrate. The shoot part is affected only after three weeks, when partial chlorosis and reduction in the rate of shoot elongation is noticed. The internodes remain underdeveloped, exhibiting a stunted growth of the plants. The leaves are reduced in size and later on, begin to dry off and ultimately the entire plant is killed. Such toxic effects of aluminium in high concentrations have also been observed by Yamano [1905] in case of wheat and rye and by Miyake [1916] in case of rice.

Manganese chloride, unlike aluminium chloride, exhibits no injury to the roots whereas harmful after-effects are to be noted in the shoot part after a fortnight (Table I). The after-effect is just of the reverse order when compared with the aluminium injury in as much as the growth of the roots continues throughout the life-cycle of the plant while the shoot portion shows retardation in its growth rate soon after the transfer. Four weeks later elongation of the shoot part is almost checked and the new leaves appear to arise from the same node, giving a more or less rosette appearance. The leaves are reduced in length and become chlorotic later on. Chlorosis and drying out of the leaves is a common feature as has been noticed by McCool [1913], McGeorge [1923], Rippel [1923] and others. By the end of the fifth week, the entire population of plants wilt and dry under the higher concentration. The severity is minimised to a certain extent in case of the lower concentration where, in spite of the death of a large number of plants by the end of the sixth week, the remaining ones are in a position to complete their life-cycle although without flowering. Relatively speaking the shoot is more elongated while the roots, though developed to a great extent, do not increase in length beyond that noted for the higher concentration.

The fact thus becomes evident that the addition of the different chlorides under higher concentration is decidedly more injurious than the lower one. This is further supported by the observations recorded in case of the lower concentration of lithium chloride which brings about a more satisfactory and vigorous growth of plants than is observed in the higher one (0·01*M*).

The case of sodium chloride is, however, different from those described above. The lower concentration in this case brings about a poor growth both of the

shoot and the root, while the higher one surpasses even the control (Table I) as regards the general growth and development of plants—a result which is in keeping with the observations of Lipman, Davis and West [1926].

In the third series of experiment the chlorides are supplied in very low concentrations of 0·0005 and 0·0001*M* strength. It is to be noted, however, that whereas the lower concentration (0.0001 *M*) is better in some respects, the relatively higher one (0·0005*M*) brings about unfavourable variations in certain cases so far as the general morphology is concerned. Cobalt chloride in 0·0005*M* concentration, though not exhibiting leaf injury and colourations characteristic of still higher concentrations, brings about malformation in roots and stunted growth of shoot, culminating in the death of plants in the third week. On the other hand, plants growing in 0.0001*M* solution survive the toxic effect and grow to ear, although the setting of grains is not well marked. The degree of toxicity of cobalt chloride, thus, goes on diminishing with its dilution in the nutrient media and although concentrations lower than 0·0001*M* have not been tested experimentally, it is probable that still lower concentrations of this salt may exert stimulative effect, as stressed by Mazé [1915] and Nakamura [1904] for certain crops.

In case of iodine chloride solution of 0·0005*M* strength the root growth is practically checked. The leaves after the first week begin to show signs of severe chlorosis and drying off—the drying proceeding from the tip downward towards the sheath. Wilting is noted in the third week. In still dilute solution of 0·0001*M* strength, the plants survive for a longer time, but the growth of root is poor while the shoot, though exhibiting vigorous growth in the beginning, shows a slight chlorosis of leaves after about a month. With further advance in age, toxicity is more pronounced and small streaks of white patches scattered all over the surface—more towards the margin, less towards the midrib—appear in this lower concentration. Iodine, thus, even in such a dilute concentration as 0·0001*M* exerts its toxic effect in contrast to the observations of Aso and Suzuki [1904-05] and Mazé [1915 ; 1919] made upon other crop plants. Brenchley [1924], on the other hand, observed that different species of plants vary in their reaction towards this element. She found that iodine badly checked the germination and growth of barley and mustard, the former being more sensitive to its toxic action ; but that if some time elapsed between the treatment and the sowing, much of the toxic action of iodine disappeared with the result that the former was affected to some extent while the latter remained unaffected, suggesting thereby that much of the iodine during this interval was removed, in some way or other, from the field of action of the plant root possibly by adsoprtion by the various constituents of the soil.

Unlike the undesirable symptoms produced under high concentration, zinc chloride in 0·0005*M* strength leads to stimulated growth of root, shoot and assimilating surface and earlier formation of ears. It exhibits its stimulating effect even under still lower concentration (0·0001*M*) (Table I) as observed by Nakamura

[1904] in certain cases. The higher concentration ($0 \cdot 0005M$) is, however, favourable more for shoot growth than for the root growth as compared to the lower one.

Aluminium chloride of $0 \cdot 0005M$ strength exhibits poor development of root although the shoot growth has been encouraging. But still lower concentration ($0 \cdot 0001M$) leads to vigorous growth of root, shoot and assimilating surface (Table I) a result quite in accord with the observations of Kratzmann [1914]. In general the dilute solution is far more beneficial than the stronger ones [McLean and Gilbert, 1928]. This effect is also apparent in case of manganese chloride where increase in root and shoot length and assimilating area over the control is characteristically noted in the $0 \cdot 0001M$ concentration. That manganese in smaller doses brings about stimulative effects, has been noticed also by Loew and Sawa [1902], Aso [1907], McHargue [1922], Samuel and Piper [1929] and others.

Lithium and sodium chlorides are found to have entirely different effects inasmuch as the relatively higher concentrations are better for plant growth. Although the range of concentration of lithium chloride best suited for plant growth is between $0 \cdot 0005M$ and $0 \cdot 005M$ strength, below and above which growth being seriously affected (Table I), no remarkable increase in growth over the control has been observed by the application of this salt—a result found in contrast to the observation of Nakamura [1904]. In case of sodium chloride, however, it is to be remarked that the stimulative effect increases as the concentration is raised higher and higher, better growth being observed under $0 \cdot 01M$ concentration as indicated above. Lipman, Davis and West [1926] working on the tolerance of plants to sodium chloride have noted more or less similar response inasmuch as the decreasing concentrations of this salt resulted in the decreasing growth of the root and shoot.

Dry weight production in plants.—Like morphological variations, the dry weight of plants also, at successive stages of plant growth, undergoes modification in response to the concentrations of the different salts used for the experimental purpose. The toxic or useful effects of the different chlorides, supplied in different concentrations, soon influence the production of dry matter and lead to greater divergence in the ascending portion of the time-weight curves (Figs. 2-8), although the initial capital (dry weight) with which the plant starts its life-cycle remains practically the same in different cases (Table I).

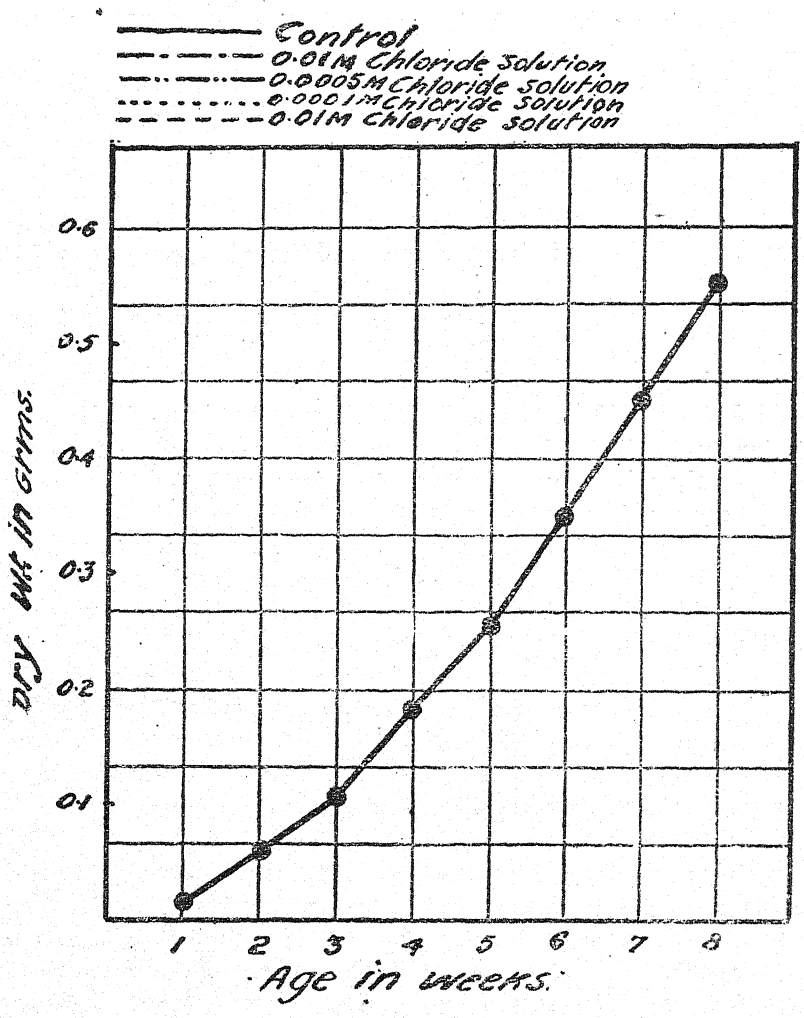
Control
0.01M Chloride solution
0.0005M Chloride solution
0.0001M Chloride solution
0.01M Chloride solution
Dry wt in grms.
0.6
0.5
0.4
0.3
0.2
0.1
1
2
3
4
5
6
7
8
Age in weeks.

Fig. 1.

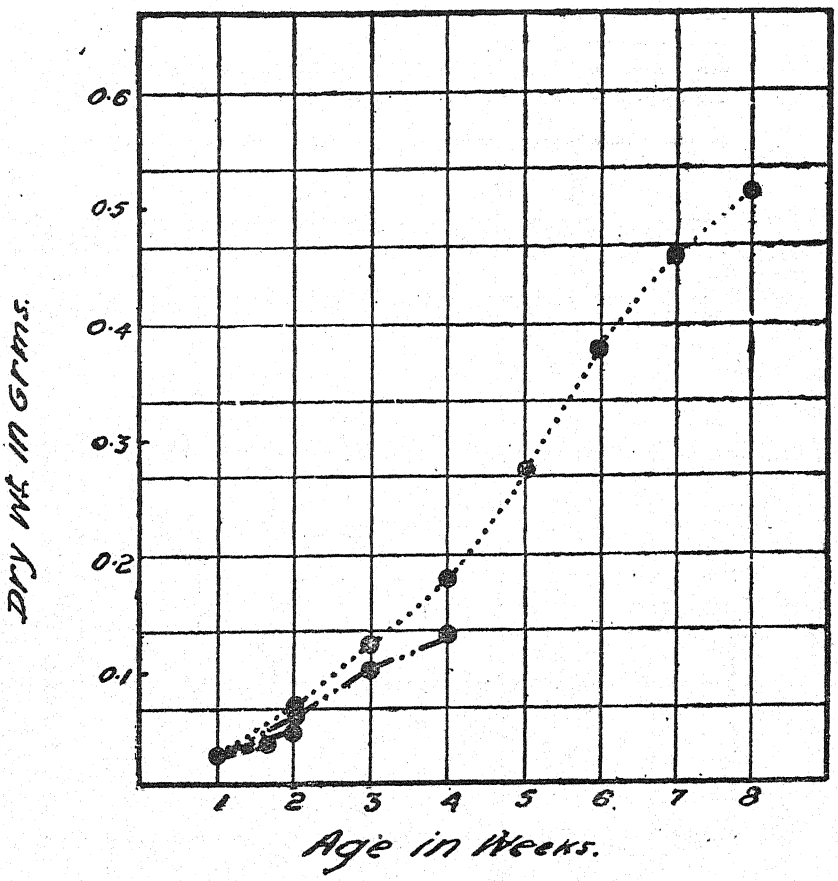

Fig. 2.

[*Please refer to the legend of Fig. 1 (p. 726).*]

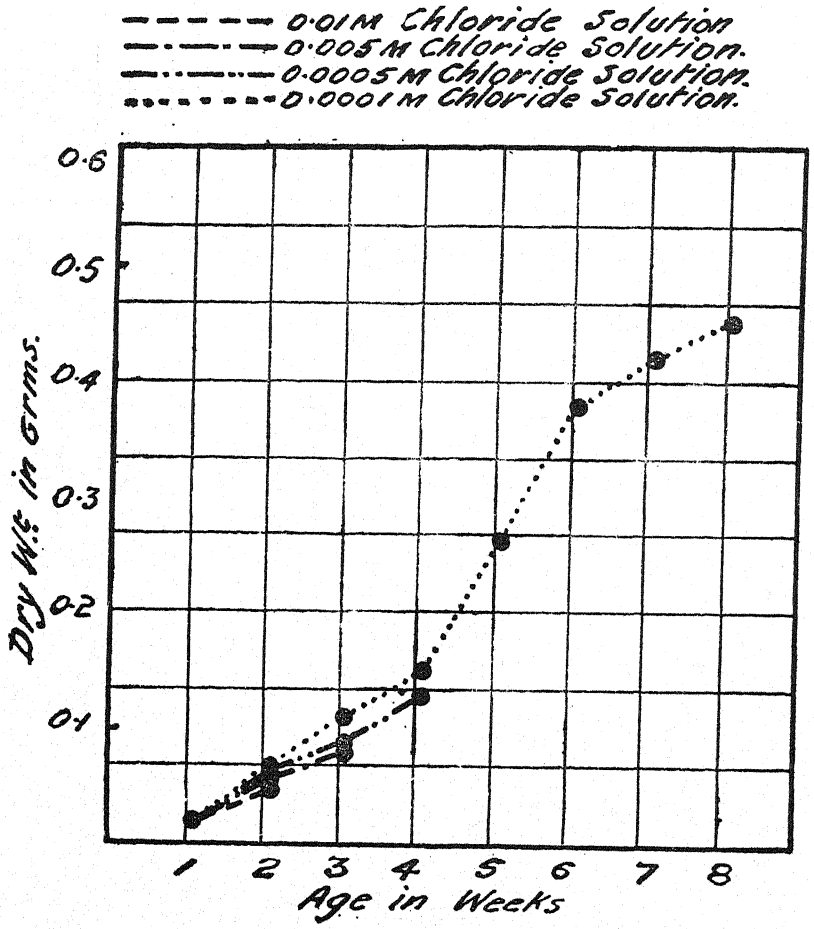
0·01M Chloride Solution
0·005M Chloride Solution.
0·0005M Chloride Solution.
0·0001M Chloride Solution.
Dry Wt in Grms.
0·6
0·5
0·4
0·3
0·2
0·1
1
2
3
4
5
6
7
8
Age in Weeks

Fig. 3.

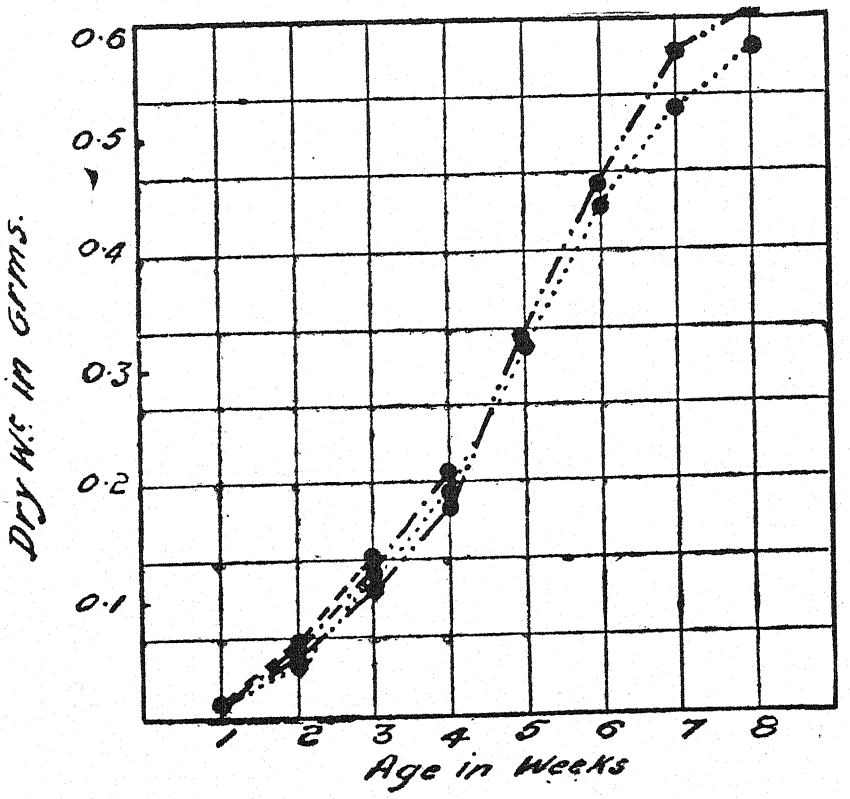

Fig. 4.

[*Please refer to the legend of Fig. 3 (p 728)*].

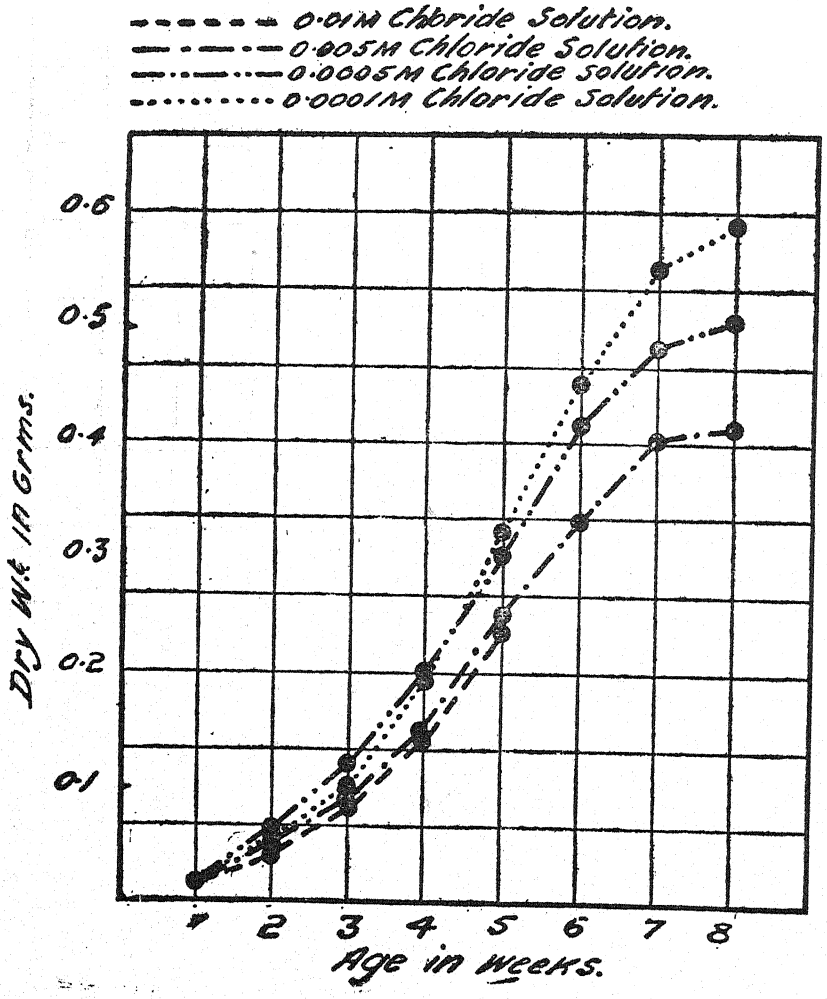
0·01M Chloride Solution.
0·005M Chloride Solution.
0·0005M Chloride Solution.
0·0001M Chloride Solution.
Dry Wt. in Grms.
0·6
0·5
0·4
0·3
0·2
0·1
1
2
3
4
5
6
7
8
Age in Weeks.

Fig. 5.

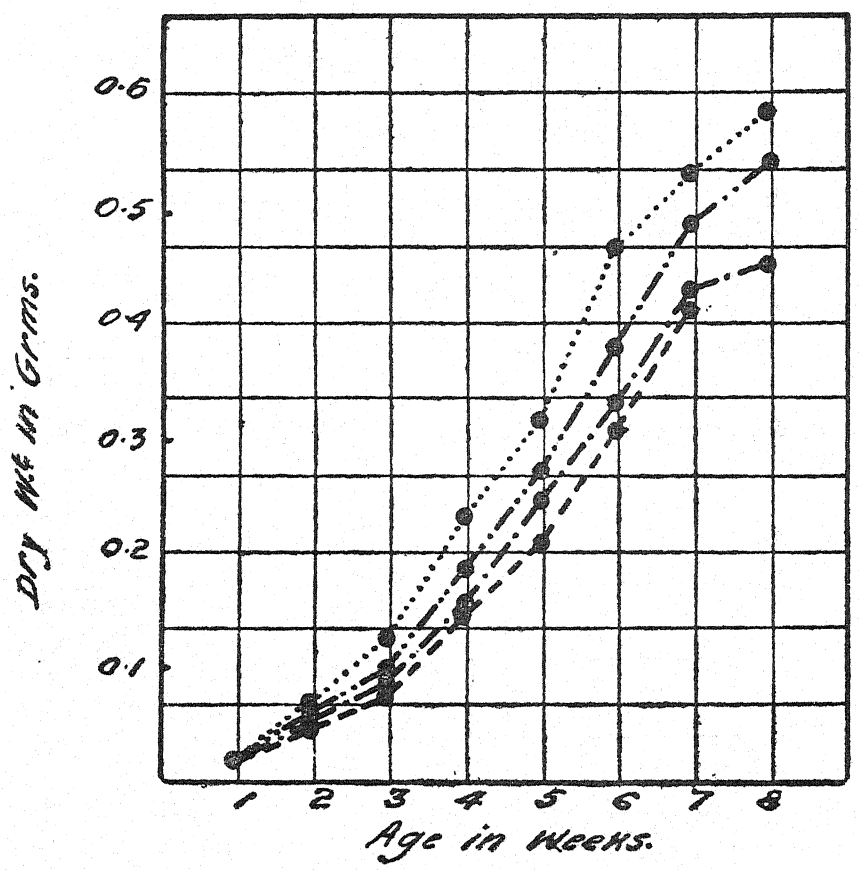

Fig. 6.

[*Please refer to the legend of Fig. 5 (p. 730).*]

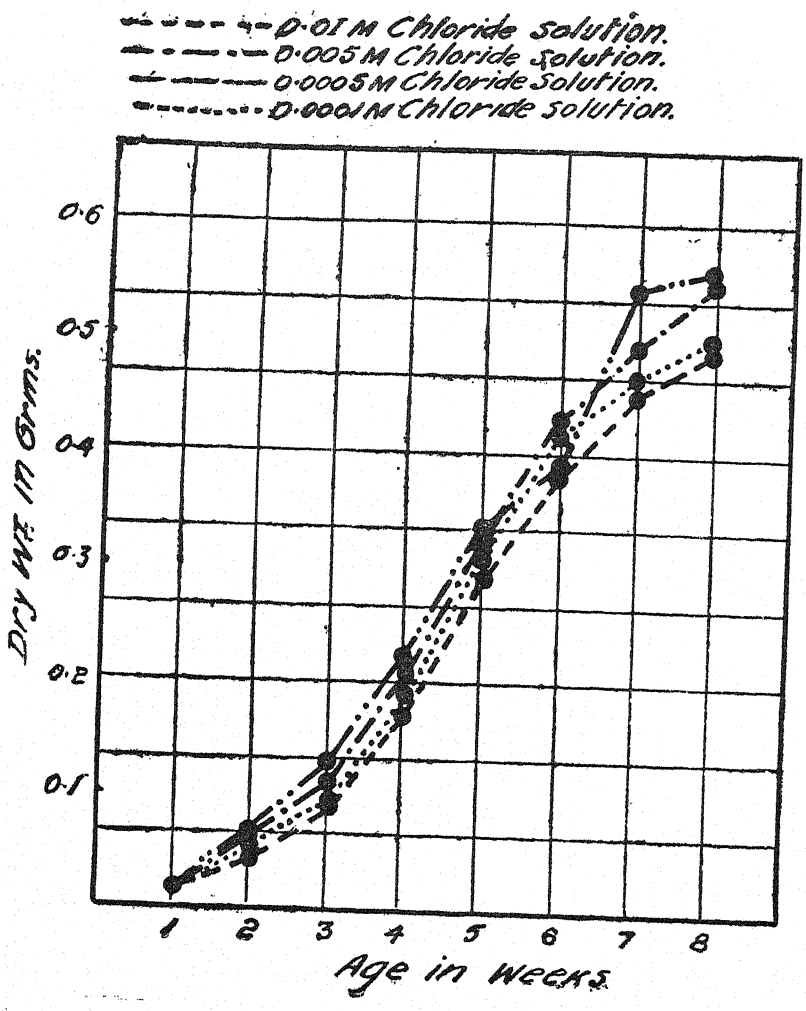
0·01 M Chloride solution.
0·005 M Chloride solution.
0·0005 M Chloride solution.
0·0001 M Chloride solution.
Dry wt in Grms.
0·6
0·5
0·4
0·3
0·2
0·1
1
2
3
4
5
6
7
8
Age in weeks

Fig. 7.

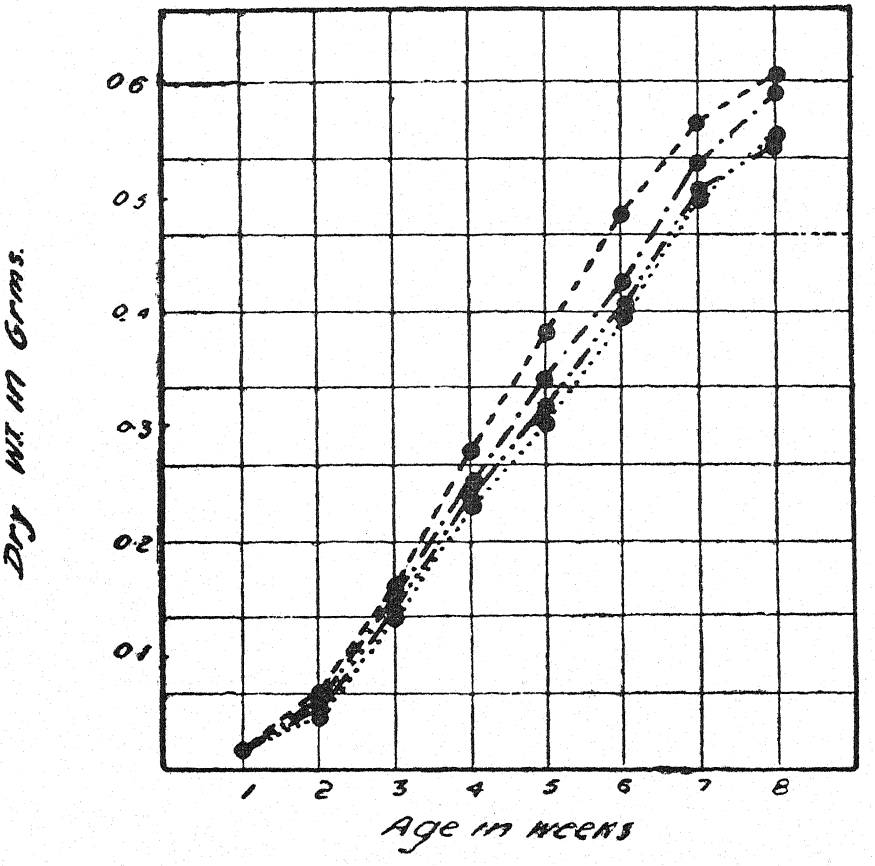

Fig. 8.

[*Please refer to the legend of Fig. 7 (p. 732).*]

While such a divergence in plants belonging to the different sets is characteristically noted in some of the salts like aluminium, manganese and zinc chlorides ; in still others like iodine, cobalt, sodium and lithium chlorides, the differentiation is not so well marked. It is to be further noted that in cases of iodine and cobalt chlorides the plants die at an early stage due to the apparently toxic effects of these salts. Especially is this true of the higher concentrations where this toxicity should naturally be more as compared to the lower ones in which case the plants are in a position to thrive better and continue to grow more vigorously for a major portion of their life-cycle. In almost all the cases, however, where the curves are complete the essential sigmoid nature is preserved (Figs. 2-8), in spite of the fact that variations have been caused in the constitution of the culture solutions. The nature of the growth reaction, thus, at successive stages remains more or less identical, but differences in intensity and magnitude of the same are to be noticed in response to the presence of the different chlorides, in the culture solutions under higher or lower concentrations.

The plants belonging to any one set although growing under differential nutritional supply do not show very significant differences in their dry weights at successive weekly stages during the early period (Table I) while with increase in the period of growth, the differences in a way become more marked at least, in certain cases where divergence in the nature of the curves has already been indicated to take place. This is all the more apparent when we take into consideration that towards the adolescent stage of plant growth photosynthentic activity markedly rises [Singh and Lal, 1935] and as such the more or less insignificant effect of these salts on the production of dry matter during early stages, becomes more significant as the age-cycle is increased.

Towards the close of the life-cycle it is to be noted that the plants growing in the nutrient media exhibit varying degrees of dry weight accumulation (Table I). In view of the observations thus collected it may be remarked that some of the culture solutions are decidedly useful in inducing greater dry matter yield whereas others exhibit only harmful effects leading to decreased dry matter formation towards the close of the life-cycle. Thus sodium, zinc, aluminium and manganese chlorides when supplied in 0·01 and 0·005, 0·0005 and 0·0001, 0·0001, and 0·0001M concentrations respectively induce greater dry matter yield which ranges between 0·5840 grm. to 0·6130 grm. in different cases (Table I) as compared to the control possessing 0·5480 grm. dry weight. At concentrations other than these the above four chlorides exhibit decidedly toxic influence inasmuch as the age-cycle is cut short as well as the dry matter yield is diminished. In contrast to these iodine and cobalt chlorides seem to have greater toxic effects and as such in none of the concentrations used, does the dry matter yield become equivalent to that obtained in the control plants, even though in certain cases the age-cycle remains the same. In lithium chloride, however, the dry matter yield under 0·0005M concentration is almost equal to that of the control.

The verdict of these statements leads us, therefore, to the view that only a few of these non-essential elements available in very dilute concentrations influence dry matter formation to some extent and as such they may be said, in general, to have very little significance of practical utility, since their presence in very small quantities in the seed or soil may be sufficient to stimulate the formation of various organic and inorganic constituents determining plant growth. To test how far the synthesis of various organic material is influenced by the availability of these elements the final chemical analysis of the plants growing in different culture solutions has been carried out with respect to the various carbohydrates (glucose, sucrose and starch) and nitrogen.

In majority of these cases the percentage of glucose does not reach to that extent as obtained in the control plants (Table II). In case of sodium chloride alone the percentage of glucose under $0{\cdot}01M$ reaches a slightly higher value than that of the control. Lithium, aluminium and manganese chlorides under $0{\cdot}0005$, $0{\cdot}0005$ and $0{\cdot}0001$, and $0{\cdot}0001M$ respectively are capable of bringing the glucose content of plants very nearly equal to that of the control. Other chlorides, however, are decidedly injurious in this direction.

As regards the sucrose content, in none of the experimental sets it is of the same order as in that of the control plants, although in certain cases, higher or lower values approaching this limit are obtained. Starch on the other hand, behaves in an entirely different manner. In majority of the sets the starch content of plants is higher than that of the control. This substance is thus capable of accumulation to a greater extent in presence of these chlorides which may thus be said to have served directly or indirectly in the increased synthesis of starch or its greater accumulation in plants.

The total carbohydrates, however, show no greater rise than the control plants in the different series of experiments except under $0{\cdot}01M$ sodium chloride, $0{\cdot}005M$ lithium chloride, $0{\cdot}0005M$ zinc chloride, and $0{\cdot}0001M$ manganese chloride. These concentrations of the above four chlorides appear to have greater stimulative effect than their other concentrations. In the cases of other chlorides the different concentrations experimented upon, appear to be toxic in their effect, bringing about a decline in the carbohydrate content of the plant tissues.

The total nitrogen, too, undergoes similar response inasmuch as, in majority of the cases its content never reaches the limit attained by the control plants except in $0{\cdot}01M$ and $0{\cdot}005M$ of zinc chloride and $0{\cdot}0001M$ of aluminium chloride.

It may be remarked here that in discussing the data on the chemical analysis of plant material the economic aspect has mainly been taken into consideration as examined by the values obtained towards the close of the life-cycle. It is, however, necessary to point out at this stage that the age-cycle in different solutions has never been a common feature throughout the entire series of experimentation. Curiously enough in certain cases, at least, the carbohydrate and nitrogen contents of the plant tissues reach the same order in the short-lived plant

as that obtained for long-lived ones and sometimes surpass the latter in this respect. It thus appears that with the shortening in the age-cycle the synthesis and accumulation of these materials start at a slightly greater rate to compensate the deficiency and to bring the plants into one level of organic and inorganic content. The entire series of chemical changes occurring in the long-lived plants thus attain similar magnitude within a shorter range, in the short-lived ones. From a physiological point of view, therefore, the rate of their formation may be greater, but from an economic point of view the final outturn never appears to be paying and as such it cannot be claimed that the majority of these elements have stimulative effect upon the chemical changes attendant upon growth, although in the very strict sense the greater rate of the synthesis is sufficient justification of their stimulative effect in these directions.

The physiological rôle of these elements thus appears to be of no definite nature inasmuch as the stimulative and beneficial effect in one direction is counteracted by the baneful effect in others and as such it becomes a difficult task to stress with any degree of certainty the exact physiological rôle of these elements.

Summary

The investigation reported in the present paper is taken up with a view to studying the effect of some of the so-called non-essential elements employed in the form of their respective chlorides. *Triticum vulgare* (*var.* Pusa 4) has been used for experimentation. The different cultures have been divided into a number of series, the first being designated as the control, while the rest include the chlorides supplied in four different concentrations ranging from 0·01 to 0·0001*M* strength. The experiments have been conducted under rigidly controlled environmental conditions. Factors other than the concentration of chlorides are kept above their limiting value such that the variations observed may wholly be accounted for on the basis of the concentration of the various chlorides used for experimental purpose. The changes introduced as a result of the application of the various chlorides have been studied in terms of certain morphological and compositional variations, *viz.*, shoot and root length, assimilating surface, dry matter production, and the different carbohydrates and nitrogen accumulation, etc. The following inferences are drawn from the observations recorded herein :—

Salts	Concentrations	Effect
$CoCl_2$	0·01*M*	Extreme toxicity ; a characteristic coloration of leaves ; plants die within thirty-six hours of application.
	0·005*M*	Severe toxicity ; plant survive for about a week after application.
	0·0005*M*	Mild toxic effect ; pl nts su vive for about three weeks after application.
	0·0001*M*	Feeble toxicity ; plants complete their life cycle with earlier ear formation but final dry matter yield is below the control,

Sal s	Concentrations	Effect
	0·01*M*	Severe toxicity ; plants die within a week after addition.
	0·005*M*	Severe toxicity leading to the death of plants two weeks after application.
:1	0·0005*M*	Lessened toxicity ; plants survive for about three weeks after application.
	0·0001*M*	Mild oxicity ; plants complete their life-cycle but the final dry matter yield is below the control.
	0·01*M*	Severe toxicity leading to the death of plants two weeks after application.
	0·005*M*	Lessened toxicity ; plants survive for about three weeks after application.
$ZnCl_2$	0·0005*M*	A remarkable stimulative effect ; plants survive for their whole life-cycle ; plant growth and final dry matter yield is far above he normal.
	0·0001*M*	Stimulative effect resulting in the vigorous growth of plants and increased final dry matter yield.
	0·01*M*	Toxic effect ; plants die four weeks after application.
	0·005*M*	Toxic effect ; most of the plants die without completing their life-cycle while the remaining ones exhibit growth and final dry matter yield below the control.
$AlCl_3$	0·0005*M*	Stimulative effect ; plants survive for their whole life-cycle but with a final dry matter yield which is below the control.
	0·0001*M*	A better stimulative effect ; the growth and final dry matter yie'd is far above the normal.
	0·01*M*	Toxic effect ; plants die before completing their life-cycle. Poor stunted growth with greatly diminished dry matter yield.
$MnCl_2$	0·005*M*	Toxic effect ; growth and final dry matter yield much below normal.
	0·0005*M*	Slight toxicity ; growth and final dry matter yield more or less equal to the normal.
	0·0001*M*	Stimulative effect ; plant growth and final dry matter yield are above the normal.
	0 01*M*	Toxic effect ; plants exhibit poor growth and final dry matter yield is below the normal.
	0·005*M*	No toxicity ; growth and final dry matter yield is more or less equal to the control.
LiCl	0·0005*M*	No toxicity ; the general vigour and final dry matter yield is almost equal to the normal.
	0·0001*M*	Retardation in growth and final dry matter yield which is below the control.
	0·01*M*	Stimulative effect. Better plant growth and greater final dry matter yield than the control.
	0·005*M*	Lessened stimulative effect. The growth and final dry matter yield is above the control.
NaCl	0·0005*M*	No stimulative influence. Final dry matter yield is more or less equal to the control.
	0·0001*M*	Slight retardation both in growth and final dry matter yield.

The accumulation of the different carbohydrates towards the close of the life-cycle varies with the type and concentration of the chloride supplied. The glucose

content in sodium chloride under 0·01*M* is greater while in lithium, aluminium and manganese chlorides under 0·0005, 0·0005 and 0·0001, and 0·0001*M* concentrations respectively, it is almost equal to that of the control. The sucrose content is in almost all cases below the control. The starch content, in majority of the sets, is higher than that in the untreated plants. The total carbohydrates in case of sodium, lithium, zinc and manganese chlorides under 0·01, 0·005, 0·0005 and 0·0001*M* respectively, is higher than that in the control.

Nitrogen content in practically all the treated plants is lower than that in the control, zinc chloride being the only exception in which case 0·01 and 0·005*M* concentrations appear to be useful.

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TABLE I

Length of root and shoot, leaf area and dry weight per plant at successive stages of growth of Triticum vulgare (var. *Pusa 4*) *in different culture solutions*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
		Lithium chloride (0·01 *M*)		
7	7·8	6.3	8·5	0·0202±0·0004
14	8·2	7·5	14.2	0·0432±0·001
21	9·4	8·15	19.8	0·0861±0·001
28	11·7	11·45	24.65	0·1683±0·005
35	13·5	13·8	28·55	0.2871±0·003
42	13·7	14·5	30·7	0·3785±0·002
49	14·8	14·6	30·7	0·4462±0·011
56	14·8	14·6	30·7	0·4802±0·015
		Lithium chloride (0·005 *M*)		
7	7·9	6·1	8·8	0·0202±0·0004
14	8·3	7·2	15·6	0·0687±0·001
21	9·1	8·8	22·4	0·1087±0·001
28	12·2	14·4	27·2	0·2052±0·005
35	13·9	18·3	30·68	0·3201±0·006
42	14·5	20·5	32·54	0·4280±0·011
49	17·2	22·6	33·4	0·4882±0·014
56	17·2	22·6	33·4	0·5430±0·015
		Lithium chloride (0·0005 *M*)		
7	7·7	6·3	8·7	0·0202±0·0004
14	8·1	8·1	16·2	0·0684±0·001
21	9·7	13·5	22·9	0·1287±0·002
28	12·8	19·3	29·5	0·2321±0·004
35	13·7	22·9	35·8	0·3387±0·006
42	14·9	23·8	40·20	0·3868±0·008
49	18·7	24·9	42·0	0·5361±0·015
56	18·7	24·9	42·0	0·5470±0·015
		Lithium chloride (0·0001 *M*)		
7	8·1	6·0	8·6	0·0202±0·0004
14	8·4	7·1	14·4	0·0587±0·001
21	9·1	11·5	21·6	0·0927±0·002
28	11·9	16·9	28·2	0·1866±0·004
35	13·2	19·5	32·6	0·3057±0·007
42	14·6	20·2	38·8	0·4108±0·011
49	17·5	21·8	40·0	0·4603±0·012
56	17·5	21·8	40·0	0·4960±0·012

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
		Sodium chloride (0·01 *M*)		
7	8·0	6·2	9·6	0·0202±0·0004
14	8·4	8·0	18·9	0·0685±0·001
21	9·8	13·7	26·7	0·1601±0·004
28	11·9	22·4	33·4	0·2785±0·006
35	13·2	24·8	38·6	0·3792±0·009
42	14·6	25·6	39·8	0·4801±0·012
49	16·8	26·8	44·5	0·5602±0·015
56	17·5	26·8	44·5	0·6034±0·016
		Sodium chloride (0·005 *M*)		
7	8·3	6·4	9·4	0·0202±0·0004
14	8·6	7·9	18·1	0·0621±0·001
21	9·4	12·8	24·3	0·1502±0·004
28	15·1	20·9	29·9	0·2504±0·006
35	16·8	23·1	35·4	0·3400±0·011
42	17·3	23·8	36·9	0·4250±0·012
49	20·8	25·2	42·7	0·5288±0·013
56	20·8	25·2	42·7	0·5860±0·014
		Sodium chloride (0·0005 *M*)		
7	8·1	6·1	9·7	0·0202±0·0004
14	8·5	7·2	18·0	0·0587±0·001
21	9·2	11·9	23·8	0·1483±0·003
28	15·7	18·6	29·2	0·2396±0·006
35	16·9	22·8	34·7	0·3107±0·008
42	18·7	23·4	35·8	0·4003±0·011
49	21·5	24·9	42·0	0·5018±0·013
56	21·5	24·9	42·0	0·5420±0·014
		Sodium chloride (0·0001 *M*)		
7	7·8	5·9	9·4	0·0202±0·0004
14	8·3	7·0	17·9	0·0584±0·001
21	9·5	12·1	23·8	0·1407±0·004
28	16·1	18·4	28·8	0·2374±0·006
35	17·2	22·3	34·2	0·3002±0·007
42	18·7	23·1	35·4	0·3946±0·011
49	21·2	24·1	41·2	0·4973±0·012
56	21·2	24·1	41·2	0·5410±0·014
		Aluminium chloride (0·01 *M*)		
7	7·7	6·1	8·6	0·0202±0·0004
14	7·7	6·6	16·5	0·0405±0·001
21	7·8	6·6	18·7	0·0812±0·002
28	7·8	6·7	18·7	0·1457±0·004
35	7·8	6·7	18·7	0·2368±0·006

(Plants were killed due to toxicity of this solution).

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
		Aluminium chloride (0·005 *M*)		
7	8·0	6·2	8·9	0·0202±0·0004
14	8·0	6·9	16·3	0·0501±0·001
21	8·1	9·2	20·1	0·0924±0·002
28	8·2	13·7	22·4	0·1532±0·004
35	8·2	15·9	22·9	0·2503±0·006
42	8·2	16·4	23·6	0·3321±0·008
49	8·3	16·8	23·8	0·4001±0·011
56	8·3	16·8	24·8	0·4120±0·011
		Aluminium chloride (0·0005 *M*)		
7	8·2	6·2	8·5	0·0202±0·0004
14	8·2	7·2	18·9	0·0681±0·001
21	8·3	9·4	25·8	0·1201±0·003
28	8·3	15·3	35·1	0·2004±0·005
35	8·4	19·1	39·2	0·3071±0·007
42	8·4	20·8	41·3	0·4172±0·011
49	8·5	22·2	42·2	0·4853±0·012
56	8·5	22·2	42·2	0·5102±0·013
		Aluminium chloride (0·0001 *M*)		
7	7·9	6·4	8·8	0·0202±0·0004
14	7·9	8·0	20·5	0·0675±0·001
21	8·0	14·8	26·2	0·1052±0·002
28	8·3	16·2	36·2	0·1957±0·005
35	8·4	19·9	40·4	0·3201±0·008
42	8·6	21·8	42·7	0·4507±0·012
49	8·7	24·0	44·0	0·5510±0·014
56	8·7	24·0	44·0	0·5910±0·014
		Manganese chloride (0·01*M*)		
7	7·7	5·8	9·7	0·0202±0·0004
14	9·2	7·1	15·8	0·0403±0·001
21	11·4	7·2	18·2	0·0687±0·001
28	15·1	7·3	19·4	0·1403±0·004
35	15·9	7·4	20·2	0·2709±0·006
42	16·2	7·5	20·2	0·3007±0·007
49	18·2	7·5	20·2	0·4100±0·011
	(Plants were killed due to toxicity of this solution).			
		Manganese chloride (0·005*M*)		
7	7·9	5·9	9·3	0·0202±0·0004
14	8·7	7·2	15·9	0·0428±0·001
21	10·8	8·4	20·3	0·0803±0·002
28	13·9	10·7	21·8	0·1521±0·004
35	15·2	10·8	22·2	0·2403±0·006
42	15·7	10·9	22·5	0·3285±0·008
49	18·2	10·9	22·5	0·4250±0·011
56	18·3	10·9	22·5	0·4480±0·012

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
		Manganese chloride (0·0005*M*)		
7	7·8	6·2	9·2	0·0202±0·0004
14	8·6	7·3	17·4	0·0489±0·001
21	13·8	9·1	26·4	0·0952±0·002
28	15·6	14·5	33·6	0·1802±0·004
35	19·3	18·9	38·9	0·2681±0·006
42	19·5	21·8	40·6	0·3783±0·009
49	21·4	24·2	42·0	0·4857±0·012
56	21·4	24·2	42·0	0·5390±0·013
		Manganese chloride (0·0001*M*)		
7	8·0	6·0	9·4	0·0202±0·0004
14	9·8	7·9	18·2	0·0598±0·001
21	14·5	13·7	28·3	0·1203±0·003
28	19·4	18·9	35·2	0·2210±0·005
35	20·8	22·7	40·4	0·3146±0·007
42	21·2	24·1	43·4	0·4638±0·012
49	24·6	25·8	43·9	0·5287±0·013
56	24·8	25·8	44·0	0·5840±0·014
		Zinc chloride (0·01*M*)		
7	7·8	6·0	8·8	0·0202±0·0004
14	7·9	6·4	11·3	0·0652±0·001
21	8·3	6·9	12·1	0·1482±0·004
	(Plants were killed due to toxicity of this solution)			
		Zinc chloride (0·005*M*)		
7	8·2	6·2	9·1	0·0202±0·0004
14	8·6	6·3	13·2	0·0601±0·001
21	9·1	7·0	15·7	0·1268±0·003
28	9·5	7·4	16·4	0·2160±0·005
	(Plants were killed due to toxicity of this solution)			
		Zinc chloride (0·0005*M*)		
7	8·0	6·9	9·4	0·0202±0·0004
14	8·6	7·8	18·8	0·0599±0·001
21	9·8	12·9	27·8	0·1187±0·003
28	11·2	20·7	34·6	0·1943±0·004
35	12·5	23·3	49·8	0·3361±0·008
42	13·1	23·7	43·8	0·4604±0·012
49	15·7	24·7	44·5	0·5764±0·014
56	15·8	24·7	44·5	0·6130±0·015

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
		Zinc chloride (0·0001*M*)		
7	7·9	6·1	8·7	0·0202±0·0004
14	8·3	7·9	17·5	0·0578±0·001
21	9·9	13·7	25·6	0·1125±0·003
28	12·4	15·9	32·8	0·1843±0·004
35	13·8	21·1	38·6	0·3201±0·007
42	14·6	22·2	42·0	0·4423±0·011
49	17·8	23·1	42·8	0·5280±0·013
56	17·8	23·2	42·8	0·5820±0·014
		Cobalt chloride (0·01*M*)		
	(Plants were killed within 36 hours in this solution)			
		Cobalt chloride (0·005 *M*)		
7	7·9	6·2	8·7	0·0202±0·0004
14	7·9	6·4	9·5	0·0361±0·0007
	(Plants were killed due to the toxicity of this solution).			
		Cobalt chloride (0·0005*M*)		
7	8·4	6·4	8·8	0·0202±0·0004
14	8·6	6·6	13·5	0·0501±0·001
21	8·7	6·6	20·2	0·0987±0·002
28	8·7	6·9	23·8	0·1262±0·003
	(Plants were killed due to toxicity of this solution)			
		Cobalt chloride (0·0001*M*)		
7	8·2	6·1	9·2	0·0202±0·0004
14	8·2	7·5	17·8	0·0632±0·001
21	8·3	13·2	25·7	0·1183±0·003
28	8·3	16·7	33·8	0·1703±0·004
35	8·4	18·9	37·4	0·2681±0·006
42	8·5	18·9	38·9	0·3786±0·009
49	8·6	20·2	39·2	0·4552±0·012
56	8·6	20·5	39·4	0·5120±0·013
		Iodine chloride (0·01*M*)		
7	8·2	5·9	8·6	0·0202±0·0004
14	8·2	6·0	10·0	0·0387±0·0007
	(Plants were killed due to toxicity of this solution).			
		Iodine chloride (0·005*M*)		
7	8·1	6·1	8·9	0·0202±0·0004
14	8·2	6·3	10·7	0·0485±0·0009
21	8·3	6·4	11·2	0·0691±0·001
	(Plants were killed due to toxicity of this solution).			

TABLE I—*concld.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
		Iodine chloride (0·005*M*)		
7	8·4	6·4	8·8	0·0202±0·0004
14	8·5	6·9	13·0	0·0512±0·001
21	8·6	7·4	20·1	0·0843±0·002
28	8·6	8·1	23·5	0·1281±0·003
	(Plants were killed due to toxicity of this solution)			
		Iodine chloride (0·0001*M*)		
7	8·3	6·2	9·0	0·0202±0·0004
14	8·3	8·6	18·0	0·0607±0·001
21	8·4	9·8	25·9	0·1102±0·003
28	8·4	17·9	34·2	0·1581±0·004
35	8·5	20·7	38·0	0·2680±0·006
42	8·5	21·5	39·8	0·3810±0·009
49	8·6	22·2	40·1	0·4205±0·011
56	8·7	22·2	40·5	0·4521±0·012
		Control		
7	8·2	6·4	9·7	0·0202±0·0004
14	8·7	7·7	18·8	0·0601±0·001
21	9·9	12·9	26·2	0·1085±0·003
8	16·8	19·1	32·8	0·1821±0·004
35	19·5	20·8	37·2	0·2532±0·006
42	20·2	22·5	38·7	0·3487±0·008
49	22·6	24·4	42·5	0·4501±0·012
56	22·6	24·4	42·8	0·5480±0·013

TABLE II

Content of carbohydrate fractions, and total nitrogen at the final stage of growth of Triticum vulgare (var. *Pusa 4*) *in different cultural solutions*

Treatment	Percentage (dry-weight basis)					
	Glucose	Sucrose	Starch	Total carbo-hydrate	Total nitrogen	Remarks*
LiCl (0·01*M*) . .	14·32	6·84	12.60	33·76	3·780	56
„ (0·005*M*) . .	15·97	7·23	15·14	38·34	3·720	56
„ (0·0005*M*) .	15·82	9·85	16·43	42·10	3·590	56
„ (0·0001*M*) .	13·94	7·64	14·20	35·78	3·680	56

* The number of this column indicates the number of days the plants grew in the different culture solutions.

TABLE II—*contd.*

Treatment	Percentage (dry-weight basis)					
	Glucose	Sucrose	Starch	Total carbo-hydrate	Total nitrogen	Remarks*
NaCl (0·01*M*) . .	17·43	10·96	15·625	44·015	3·306	56
,, (0·005*M*) .	16·15	8·84	15·42	40·41	3·306	56
,, (0·0005*M*) .	15·63	9·62	13·21	38·46	3·421	56
,, (0·0001*M*) .	14·42	8·57	11·88	34·87	3·389	56
$AlCl_3$ (0·01*M*) .	15·35	5·27	7·93	28·55	3·502	35
,, (0·005*M*) .	15·86	8·20	11·03	35·09	3·750	56
,, (0·0005*M*) .	16·12	9·87	13·87	39·86	3·680	56
,, (0·0001*M*) .	16·48	10·62	13·65	40·85	3·890	56
$MnCl_2$ (0·01*M*) .	12·29	6·48	14·12	33·25	3·783	49
,, (0·005*M*) .	13·85	7·93	15·42	37·20	3·738	56
,, (0·0005*M*) .	14·89	10·21	13·49	38·59	3·649	56
,, (0·0001*M*) .	16·57	10·94	14·24	41·75	3·812	56
$ZnCl_2$ (0·01*M*) .	12·29	6·82	14·20	33·31	4·587	21
,, (0·005*M*) .	31·85	5·79	10·00	29·64	4·672	28
,, (0·0005*M*) .	14·88	11·76	14·84	41·48	3·685	56
,, (0·0001*M*) .	14·20	10·12	13·89	38·21	3·703	56
$CoCl_2$ (0·01*M*) .	8·21	9·19	7·00	24·40	3·515	2
,, (0·005*M*) .	11·38	8·01	12·50	31·89	3·094	14
,, (0·0005*M*) .	13·57	7·15	10·87	31·59	3·421	28
,, (0·0001*M*) .	14·92	11·94	13·48	40·34	3·613	56
ICl . (0·01*M*) .	8·63	8·12	11·02	27·77	3·248	14
,, (0·005*M*) .	10·41	7·02	16·50	33·93	2·956	21
,, (0·0005*M*) .	14·12	6·60	9·31	30·03	2·876	28
,, (0·0001*M*) .	15·05	6·95	9·02	31·02	3·051	56
Control . . .	16·58	11·16	13·50	41·24	3·813	56

* The number of this column indicates the number of days the plants grew in the different culture solutions.

A BIOCHEMICAL STUDY OF THE PADDY SOILS OF BENGAL WITH SPECIAL REFERENCE TO THEIR NITROGEN-FIXING CAPACITIES

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Fertilisation of rice fields is seldom practised in Bengal; yet the crop grows here consistently year after year without showing any change in the fertility of the soil. Obviously, therefore, there is the natural recuperation of soil nitrogen. Howard [1924] suggests that the abundant growth of algal film very often observed on the surface of the water-logged paddy fields, working conjointly with the bacteria within it, may fix certain amount of nitrogen. Experiments in this laboratory [De, 1934] have shown that when water-logged soil is exposed to sunlight, a considerable amount of nitrogen is fixed. This, however, will form the subject of a future communication. The present investigation is undertaken to study the fixation of nitrogen in the paddy soils under dry cultivated conditions.

Experimental

Six soil samples, representative of paddy soils in Bengal, were selected for the present work. They were obtained from the unmanured paddy fields of the following districts—Barisal, Birbhum, Dacca, Faridpur, Rajshahi and Rangpur. Soils from Dacca and Faridpur were locally collected while the remaining four were kindly supplied by the respective District Agricultural Officers.

Samples (0 in.—6 in.) were air-dried, passed through one-mm. sieve and then preserved in stoppered glass containers for use in the following experiments. A short description of the type of the soil, the pH and the C : N ratio are given in Table I.

Table I

Soil	Type	C : N	pH
Barisal	Deltaic	8·8	6·66
Birbhum	Lateritic	10·3	7·26
Dacca	Lateritic	9·3	5·11
Faridpur	Alluvial	10·3	8·19
Rajshahi	Calcareous	8·8	8·35
Rangpur	,,	10·2	6·36

NITROGEN-FIXING CAPACITIES OF THE SOILS

In the present experiment the nitrogen-fixing capacities of the above six soils were determined by the use of both large silica gel plate method of Winogradsky [1926] and the solution method. In the latter method the cultures are generally incubated for a long period. Cainey and Briscoe [1933] have shown that the differences in the relative nitrogen-fixing capacities of soils, as determined by the amount of nitrogen fixed in culture media, can be detected only when the incubation period is relatively short; on prolonged incubation these differences disappear. It is therefore, necessary first of all to select a period of incubation suitable for determining the nitrogen-fixing capacity of soils by the solution method.

Hundred-c.c. lots of Ashby's medium, after sterilisation, were inoculated with one-gram portions of Barisal, Faridpur and Rajshahi soils which are known to contain *Azotobacter*. The cultures were incubated at 33°C. and at the end of 4th, 5th and 7th day, the amount of nitrogen fixed were determined. The results are given in Table II.

TABLE II

Showing the effect of varying lengths of incubation period on the fixation of nitrogen in the culture solution

Soil	Period of incubation		
	4 days	5 days	7 days
Barisal	5·6	6·4	6·9
Faridpur	6·2	7·5	8·6
Rajshahi	7·6	8·2	8·7

The figures represent milligrams of nitrogen fixed and are the averages of three determinations.

It will be seen that although marked differences in the nitrogen-fixing capacities of the three soils were observed after an incubation period of four days, these differences gradually faded as the incubation was prolonged with the result that after seven days the differences between Faridpur and Rajshahi soils entirely disappeared. It was, therefore, decided to use an incubation period of four days in all studies on the fixation of nitrogen with solution method.

The nitrogen-fixing capacities of the above soils are given in Table III.

TABLE III

Showing the nitrogen-fixing capacities of the soils when tested by (1) silica gel plate method, (2) solution method

Soil	Mg. of N fixed per plate	Mg. of N fixed in 100 c.c. of solution
Faridpur	9·9	6·2
Rajshahi	8·5	7·7
Barisal	5·3	5·6
Birbhum	1·3	Very small
Rangpur	1·3	" "
Dacca	1·2	" "

FIXATION OF NITROGEN IN SOIL

The results recorded in Table III while indicating the relative activities of the nitrogen-fixing flora of different soils, give absolutely no idea as to the amount of nitrogen that may be fixed in the soil itself under natural conditions. Apart from the presence or absence of active nitrogen-fixing organisms, the fixation of nitrogen in soil is controlled by other factors such as the supply of available phosphate or nitrogen, reaction of the soil and the competition amongst the organisms for available energy. The object of the following experiment was to find out whether measurable amounts of nitrogen are fixed in the soils when they are kept under optimum conditions of temperature and moisture. It will be noted that these conditions correspond to those prevailing in the paddy fields during the winter months when the soils remain dry and fallow. A study of the effect of the addition of plant residues on the fixation of nitrogen in paddy soils was considered important as the roots, stubbles and a portion of the straw remaining in the fields after harvest, might stimulate the growth of the nitrogen-fixing organisms, being used up as energy materials. The effect of the addition of lime and phosphate on the fixation of nitrogen was also studied. The details of the experiment are as follows :

Three 120-grm. portions of each of the air-dried soils were distributed in three beakers of 400 c.c. capacity which then received the following treatments. :—

(1) No treatment (120 grms. of soil).

(2) As (1) + 0·1 grm. K_2HPO_4 + 0·1 grm. $CaCO_3$.

(3) As (2) + 2 grms. of powered rice straw.

After the contents of each beaker were thoroughly mixed, the moisture was adjusted to eighteen per cent on air-dry basis. The beakers were then put in the incubator at a temperature of 33°C. Every alternate day the loss of moisture was made up and the soil thoroughly stirred to facilitate aeration. Total nitrogen was determined at intervals of twenty, forty and sixty days. The results are given in Table IV.

TABLE IV

Showing the amounts of nitrogen fixed in different soils

(Milligrams of nitrogen in 10 grms. of dry soil)

Soil	At start			After 20 days			After 40 days			After 60 days			Mg. of N fixed in 60 days		
	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S
Faridpur . .	6·4	6·4	7·2	6·9	6·9	7·7	6·9	6·9	7·7	6·9	6·9	7·7	0·5	0·5	0·5
Dacca . .	8·3	8·3	9·0	8·3	8·6	9·1	8·6	8·6	9·4	8·9	8·9	9·6	0·6	0·6	0·6
Barisal . .	13·1	13·1	13·9	13·1	13·1	13·8	13·1	13·2	14·0	13·3	13·4	14·0	0·2	0·3	0·1
Rangpur . .	9·3	9·3	10·1	9·0	9·0	9·9	9·3	9·3	10·4	9·5	9·6	10·3	0·2	0·3	0·2
Rajshahi . .	10·3	10·3	11·1	10·3	10·4	11·2	10·4	10·4	11·3	10·3	10·4	11·3	0·0	0·1	0·2
Birbhum . .	5·6	5·6	6·4	5·6	5·5	6·5	5·6	5·6	6·5	5·6	5·5	6·5	0·0	—0·1	0·1

O = No treatment. Ca+P = Treated with phosphate and lime. Ca+P+S = Treated with phosphate, lime and straw.

TABLE V

From these results the amounts of nitrogen fixed in pounds per acre are calculated, assuming that the weight of soil per acre up to a depth of 6 in. is 2,000,000 lbs.

Amounts of nitrogen fixed in different soils expressed as lbs. per acre

Soil	Control	Ca + P	Ca + P +S
Dacca	120	120	120
Faridpur	100	100	100
Barisal	40	60	20
Rangpur	40	60	40
Rajshahi	0	10	40
Birbhum	0	—10	10

In the interpretation of the above results, difference more than 0·14 mg. of nitrogen in ten grms. of soil or twenty-eight lbs. in an acre was considered significant.

From the foregoing observations the following facts emerge :—

(1) The amounts of nitrogen fixed in the first four soils are more than the average amount of nitrogen removed annually by the crop. (28 lbs. per acre).

(2) The addition of lime and phosphate in the soil had no marked effect on the fixation of nitrogen. This shows that the lack of available phosphate or the unfavourable soil reaction are not the factors inhibiting the fixation of nitrogen in these soils.

(3) Excepting in Rajshahi and Barisal soils, the addition of straw had no effect on nitrogen fixation. In the former soil, it has stimulated nitrogen fixation but in the latter its effect was depressive.

The results in Tables III and V show that the nitrogen-fixing capacity of a soil, as determined by the silica gel plate method or solution method, may give an entirely wrong idea about the actual fixation of nitrogen in the soil itself under natural conditions. For example, Rajshahi soil fixed the largest amount of nitrogen in culture solution and Dacca soil the least but their positions were reversed in Table V. The addition of straw did not bring about an increased fixation of nitrogen in most of the soils, indicating that the energy material already present was sufficient. Some soils such as Rajshahi and Birbhum, in spite of the conditions being made favourable for nitrogen fixation by the addition of phosphate, lime and straw, did not show much improvement in the fixation of nitrogen. It would thus appear that certain soils, even when harbouring beneficial flora, *e.g.*, *Azotobacter*, may become permanently inactive in fixing nitrogen and that treatment of such soils is not likely to improve their nitrogen-fixing power. Factors responsible for such infertility of the soil towards the nitrogen-fixing flora are, however, unknown

at present. The fixation of large amount of nitrogen in Dacca soil which does not contain *Azotobacter* indicates the possibility of existence in paddy soils of very active nitrogen-fixing organisms which have not yet been discovered.

Influence of sunlight on the fixation of nitrogen in soil

In the previous experiment on the fixation of nitrogen in the paddy soils, one important factor has been ignored, namely the influence of sunlight on the fixation of nitrogen in soil. A study of this factor is important inasmuch as some workers have claimed that the blue green algae of soil are capable of fixing atmospheric nitrogen either independently or in symbiosis with nitrogen-fixing organisms. As the influence of algae on the fixation of nitrogen was not manifest in the above experiment due to the incubation of the soil in the dark (incubator), it was proposed to conduct another experiment to see if, on incubating the soil in the light and thus facilitating the growth of algae, the fixation of nitrogen in the soil could be increased. For this purpose 100-grm. portions of Faridpur soil were taken in a number of beakers with covers some of which were thickly painted outside by Japan-black. The moisture content of the soil was maintained at eighteen per cent throughout the experiment. The results of nitrogen determinations showed that there is no significant difference in fixation of nitrogen in the two treatments after exposure to sunlight for a month.

Influence of water-logging on the nitrogen-fixing capacity of soil

As rice fields remain submerged for a long period during the growth of the crop, it was thought that water-logging might profoundly modify their nitrogen-fixing capacities by altogether destroying the strictly aerobic nitrogen-fixing organism and by encouraging the growth of those thriving well under anaerobic conditions. To investigate this point, the following experiment was done in which the nitrogen-fixing capacity of dry cultivated soil was compared with that of the corresponding water-logged soil by the solution method using an incubation period of four days.

One hundred grm. of Faridpur soil were placed in a beaker and incubated at 33°C. after the moisture content had been adjusted to eighteen per cent on dry basis. The soil was frequently stirred to facilitate gas exchanges and the loss in moisture was made up by the periodic additions of water. At different intervals, samples were withdrawn by a sterile cork borer and after having determined the moisture contents, one-gram portions of soil on dry basis were inoculated into each of three flasks containing 100 c.c. of Ashby's mannite solution. After four days' incubation at 33°C., the contents of the flasks were analysed for total nitrogen. For the determination of the nitrogen-fixing capacity of the water-logged soil, a number of test-tubes each containing one gram of soil on dry basis and 5 c.c. of distilled water were incubated at 33°C. For each experiment, three test-tubes were removed and the contents, after thorough shaking, were separately poured

into three flasks each containing 100 c.c. of mannite solution. Total nitrogen was determined as before after four days' incubation. The results show that the activities of the nitrogen-fixing organisms of soil were depressed to some extent under water-logged conditions.

TABLE VI

Showing the effect of water-logging on the nitrogen-fixing capacity of soil. Figures represent milligrams of nitrogen fixed in 100 c.c. of culture solution.

Days after incubation	Dry cultivated soil	Water-logged soil	Difference
3	5·8	5·0	0·8
10	7·6	5·8	1·8
15	8·0	6·0	2·0
20	8·5	6·3	2·2

Figures are the averages of three flasks.

BACTERIOLOGICAL EXAMINATION OF THE SOILS

The fixation of nitrogen in ordinary arable soils is supposed to be the result of the activities of *Azotobacter* and *B. amylobacter* which commonly occur in soils and are considered to be very active nitrogen fixers. There is another group of nitrogen-fixing organisms known as *B. radiobacter* which is present in soil in so great abundance that its role in the fixation of nitrogen in soil cannot be overlooked. But in the paddy soils, because of the swamp conditions prevailing for about half the year, the fixation of nitrogen might not be brought about by the same agents as those in normal soils. Accordingly the determination of the relative abundance of the above three groups of organisms in different soils was made to find out whether the amounts of nitrogen fixed were related to the number of nitrogen-fixing organisms in those soils. The following methods were used for counting the organisms :—

Azotobacter.—The method is essentially the same as that described by Winogradsky [1926], the only difference being that agar plates were used in place of silica gel plates. It has been shown by Curie [1932] that result obtained with agar plates were sufficiently uniform to permit a quantitative comparison of the *Azotobacter* flora of different soils.

B. radiobacter.—The method originally devised by Smith [1928] and subsequently modified by Starky [1929] was used for counting the number of *radiobacter*. In the original method of Smith, the diluted soil suspension (100 c.c.) is treated with 0·5 c.c. of 0·1 per cent crystal violet and one-c.c. portions of the suspension are plated out. Starky's modification consists in adding the dye (one c. c. of 0·1 per cent solution) to 100-c. c. portions of the medium immediately before plating. It may be noted that the concentration of dye in Starky's medium

is ten times greater than in Smith's. The advantage of Starky's method is that the colonies appearing on the plates are mostly of *B. radiobacter*, which can be easily distinguished from the contaminating growth.

B. amylobacter.—The number of *B. amylobacter* in soil was determined by dilution method adapted from one in use in the General Microbiological Department of the Rothamsted Experimental Station. The method depends on setting up duplicate cultures containing one c.c. of each of a series of thirteen half-and-half dilutions of a well-shaken soil suspension of known strength in 15 c.c. of a nitrogen-free agar medium [No 11, Waksman and Fred, 1928]. After incubating anaerobically at 33°C. for a week, the cultures were examined. By noting the number of cultures from which the organism is absent it is possible to estimate statistically the number of orgnisms per gram of soil. The statistical tables constructed by Fisher [1922] were used for this purpose. The following table gives a detailed record of the cultures made from Faridpur soil in order to illustrate the method of setting up the cultures for the purpose of obtaining information about the frequency of the organism in soil.

TABLE VII

Degree of dilution of soil suspension	Culture number	Growth
1 : 50	1	+
	2	+
1 : 100	1	+
	2	+
1 : 200	1	+
	2	+
1 : 400	1	+
	2	+
1 : 800	1	+
	2	+
1 : 1600	1	+
	2	+
1 : 3200	1	+
	2	+
1 : 6400	1	+
	2	+
1 : 12800	1	—
	2	—
1 : 25600	1	+
	2	—
1 : 51200	1	—
	2	—
1 : 102400	1	+
	2	—
1 : 204800	1	—
	2	—

Number of sterile tubes=8.

Number of bacteria per gram of soil=10,600.

(From Fisher's table).

TABLE VIII

Showing the number of nitrogen-fixing organisms per gram of soil

Soil	Azotobacter colonies	*B. amylobacter*	*B. radiobacter*
Faridpur	1310	10,600	48,125
Rajshahi	2740	23,000	34,375
Barisal	144	118,000	5,000
Birbhum	Nil	2,600	25,000
Rangpur	,,	22,000	Very few
Dacca	,,	32,000	Nil

SEARCH FOR NEW NITROGEN-FIXING ORGANISMS

It has already been pointed out that the organisms fixing nitrogen in the paddy soils might not be the same as those occurring normally in ordinary dry cultivated soils. Justification for such an assumption is further obtained from the fact that Dacca soil fixed the greatest amount of nitrogen (Table V) in spite of its low content of nitrogen-fixing flora as compared with those in Faridpur, Barisal and Rajshahi soil (Table VIII). Accordingly a search was made for the presence of new nitrogen-fixing organisms in all the six soils used in the present investigation. For isolation of the organisms both Winogradsky's nutrient silica gel plates and Ashby's agar plates were inoculated with small particles of soil. The plates were then incubated at 33°C. and examined daily. After six days' incubation it was noticed that a large number of raised and transparent colonies were appearing along with those of *Azotobacter* in the plates inoculated with Faridpur soil. The colonies were so tenacious that a part of it could not be removed by rubbing with platinum needle. Microscopic examination of the growth showed the presence of very large, encapsulated, spore-forming rods. For purification, a little growth was boiled with sterile water for two minutes and the hot suspension plated on Ashby's agar. In about three days, raised transparent colonies appeared all over the plates. The process was repeated once more to ensure the purity of the culture. The organism made very good growth on Ashby's agar plate but the growth in Ashby's solution was relatively much poor. The organism which has the following characteristics does not appear to have been described in Bergey's manual.

1. Large encapsulated rods generally occurring in packets; Length 5.6μ with the capsule and 1.5μ without capsule.
2. Gram-positive and non-motile.
3. Spore-forming.
4. Strictly aerobic.
5. Nitrate reduced to nitrite.

6. Formation of gas and acid in nitrogen-free glucose medium.
7. Milk curdled.
8. Broth slightly turbid.
9. Fair growth on potato, colonies greyish brown in colour.
10. Amount of nitrogen fixed on agar plate for every gram of mannite decomposed—5 mgs.

Dacca soil.—On both silica gel plates and Ashby's agar plates a large number of milk-white, raised and opaque colonies appeared after three week's incubation. A little growth was microscopically examined and was found to consist of spherical cells occurring in pairs—resembling in appearance the typical *Azotobacter* cells. The organism is non-motile and does not produce spore or pigment. It is stained only with difficulty by aniline dyes. In spite of various attempts the organism could not be obtained in pure culture. It seems to be a new species probably belonging to the genus *Azotobacter*.

Rajshahi and Barisal soils—The only important growth on the plate was that of *Azotobacter*.

Birbhum soil:—There was very little growth on silica gel plate.

Summary

1. Fixation of nitrogen was studied with six paddy soils obtained from different districts of Bengal.

2. Amounts of nitrogen fixed in four of the soils were more than the amount removed annually by the crop.

3. The addition of phosphate or lime in the soil had no marked effect on nitrogen fixation.

4. On adding straw, nitrogen fixation was stimulated only in one of the soils.

5. Sunlight has no effect on the fixation of nitrogen in soil.

6. Water-logging was found to inhibit the activities of the nitrogen-fixing organisms of soils.

7. The number of nitrogen-fixing organisms in different soils were counted. They were present in large number in most of the soils.

8. A new nitrogen-fixing organism was isolated from Faridpur soil.

9. In determining the nitrogen-fixing capacity of soil by solution method, differences in the nitrogen-fixing abilities can be detected when the incubation period is relatively short (four days).

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BISMUTHATE METHOD FOR THE ESTIMATION OF MANGANESE IN PLANT MATERIAL

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Since the recognition of the important role played in the metabolism of the plant by elements which occur in relatively small amounts the so-called "non-essential elements"—the attention of the analytical chemist has been drawn to the elaboration of methods for their accurate determination. Since these elements, though present in very minute amounts, are regarded as necessary for the proper growth and development of the plant and as important as those which constitute the greater portion of it, it has been felt that the methods used for their estimation must be sensitive and accurate enough to yield reliable results under a wide range of conditions. Manganese is one of these elements. The importance of manganese in increasing the oxygen-carrying power of oxidases was first recognised by Bertrand [1897], McCool [1913]; and Mclean and Gilbert [1925] explain chlorosis as the result of the absence or deficiency of manganese. McHargue [1922] regards manganese as being concerned in nitrogen assimilation and protein synthesis and also to function with iron in the synthesis of chlorophyll.

In view of the important role played by manganese in plant metabolism, it is essential to have a reliable method for its accurate determination, specially when it is present in small amounts. In the literature there are mentioned a large number of methods; colorimetric, gravimetric and volumetric, which have been advocated by different workers and used from time to time for the estimation of this element. Colorimetric methods, although capable of giving fairly accurate results, suffer from the serious disadvantage that all workers are not equally sensitive to slight changes in the intensity of tints. Gravimetric methods are believed to yield accurate results. This accuracy, however, diminishes when the elements to be estimated are present in minute quantities. In such cases, even an otherwise slight error, due to the solubility of precipitates or its contamination with impurities is greatly magnified. The volumetric methods are considered to be free from such defects and in case a satisfactory method for any particular estimation is available, it is to be preferred.

Schneider [1897] was the first worker to propose a volumetric method for the estimation of manganese. It was based upon the use of lead peroxide as an oxidising agent. Ibbetson and Brearley [1901], with certain modifications report to have successfully used this method in the analyses of white metal alloys. It was however, not before 1904, that the method was recommended for general application by Blair, who studied it in detail. Davidson and Capen [1931] made a comparative study of this, and the persulphate and periodate colorimetric methods as well as the gravimetric method. They found that of all the above methods the sodium bismuthate method gave the lowest results, exceptionally high figures being obtained with the gravimetric method. For the estimation of manganese in soils the bismuthate method has been in use in these laboratories for a number of years and has always given satisfactory results.

The suitability of this method for the analysis of plant material, as compared with the gravimetric method of the A. O. A. C. [1925], has been studied under a wide range of well-controlled conditions and it has been found that with some modifications in the technique proposed by Blair [1904] for the analysis of iron and manganese ores, the method gives satisfactory results.

Method

Preparation of extract

Finely ground plant material to the extent of 200 grams is carefully ignited at a dull red heat as completely as possible. The ash obtained is digested with strong hydrochloric acid for eight hours on a sand-bath, at a temperature just sufficient to cause occasional ebullition. When digestion is complete, the residue in the conical flask is transferred to a porcelain dish and evaporated to dryness on a water-bath, thus rendering silica insoluble. The contents of the dish are now extracted with hydrochloric acid, filtered through a hardened filter paper, washed with distilled water till free from chlorides and the filtrate made up to one litre. We prefer this method of preparing the extract to the one in which plant material is ashed separately for the estimation of each element or group of elements, in as much as the errors due to individual variations of sampling, which will be reflected in the ratios of different elements determined are eliminated when a large amount of the sample is taken, ashed and extracted in one lot; also it saves much time, which is otherwise taken in ashing and extracting individual samples separately. Further, the ash obtained can be stored over any length of period without undergoing any change in its composition whereas the plant material is subject to attacks by insects and fungus and thus liable to changes in composition.

Estimation of manganese

Aliquot portions from the stock solution prepared as above are transferred to porcelain dishes, a few drops of concentrated nitric acid added and the whole

evaporated to dryness on the water-bath. The residue in the dishes is now cooled, and after the addition of 2·5 c.c. of concentrated sulphuric acid is again heated on sand-bath till copious fumes of SO_3 begin to be evolved. This removes all traces of hydrochloric acid. The dishes are now cooled and the contents transferred to a conical flask of 300 c.c. capacity, with the aid of 50 c.c. of approximately 4 *N* nitric acid.* In order to assist solution, the liquid in the flasks is heated to boiling, care being taken to avoid loss of acid as far as possible. The temperature of the liquid is now brought to within 15° to 20°C., which after the addition of 0·5 gram of sodium bismuthate (B. D. H., A. R. preparation) is kept aside for half an hour and occasionally shaken. After keeping it undisturbed for about five minutes, fifty c.c. more of three per cent nitric acid, also free from nitrous acid, are added ; taking care that the temperature during the operation does not exceed 30°C. The solutions are now filtered through a mat of specially prepared asbestos(*), spread over a perforated porcelain disc fitted in a glass funnel and the residue washed with fifty c.c. of the same three per cent nitric acid, using small quantites at a time. The filtration and washing does not take more than five minutes. The resulting permanganate is reduced by adding a known volume of approximately *N*/100 ferrous ammonium sulphate solution (containing 50 c.c. of concentrated sulphuric acid per litre of solution) and the excess of ferrous salt is immediately titrated with *N*/100 potassium permanganate. A blank determination using the same amount of sodium bismuthate and nitric acid solution is also carried out simultaneously. The difference between the amount of centinormal permanganate solution used for the two titrations gives the amount of manganese present ; one c.c. of *N*/100 $KMnO_4$ corresponds to 0·000153 gram of Mn_3O_4.

Results

1. *Estimation of manganese in the plant ash extract by the gravimetric and the bismuthate methods*

(*a*) It will be seen from the results given below in Table I that the amount of manganese determined by the gravimetric method is about eight times as great, although duplicate determinations carried out on different quantities of the stock solution by both the methods give results which agree fairly closely amongst themselves.

* The nitric acid should be prepared from strong acid which has been freed from all traces of nitrous fumes by passing a current of air through it, which can be conveniently done with the help of a water pump.

(*) The asbestos used for this filtration is first digested with five per cent NaOH, washed with water, again digested with HNO_3 (1 : 4) and then washed until free of all traces of acidity. It is then dried, ignited and made into a suspension with wate .

TABLE I

Showing the amount of manganese obtained by the gravimetric and bismuthate methods

Volume of extract taken	Gravimetric method		Bismuthate method	
	Amount of manganese obtained as Mn_3O_4	Per cent Mn_3O_4 on oven-dried material	Amount of manganese obtained as Mn_3O_4	Per cent Mn_3O_4 on oven-dried material
25 c. c. .	0·0033	0·066	0·000428	0·0085
50 c. c. .	0·0067	0·067	0·000841	0·0084

(*b*) In order to test further, the relative accuracy of the two methods for estimating small quantities of manganese, an artificial plant extract containing known amount of manganese was prepared by adding small quantities of hydrochloric acid, ferric chloride, magnesium sulphate, calcium chloride, potassium phosphate and alum, etc. to a solution of manganese sulphate (0·7 grm. per litre). The figures for the manganese obtained by the two methods, are given below in Table II.

TABLE II

Determination of manganese in a solution containing known quantity of $MnSO_4$ in the presence of other salts

(10 c.c. of the solution=0·00354 grams of Mn_3O_4 were used for estimation)

Mn_3O_4 present in the solution	Gravimetric method		Bismuthate method	
	Amount of Mn_3O_4 as determined	Per cent of the the amount taken	Amount of Mn_3O_4 as determined	Per cent of the amount taken
0·00354	0·0060	170	0·00359	101·4
0·00354	0·0058	164	0·00359	101·4
0·00354	0·0056	158	0·00359	101·4

The results obtained by the gravimetric method are much higher than those by the bismuthate method which agree very closely with the amounts taken.

2. Recovery of added manganese

This was tested by adding to the extract known quantities of manganese in the form of *N*/100 potassium permanganate and deducting from the total amount determined the quantities present in the original extract as had been determined previously by the two methods respectively. The following results were obtained :—

TABLE III

Recovery of added manganese

Volume of plant extract taken for estimation.	Amount of manganese added as $N/100$ $KMnO_4$	Gravimetric method			Bismuthate method		
		Total Mn_3O_4 determined in the mixture	Mn_3O_4 recovered (after deducting the amount previously determined by this method)	Recovery per cent. of Mn_3O_4 added	Total Mn_3O_4 determined in the mixture	Mn_3O_4 recovered after deducting the amount previously determined by this method	Recovery per cent of Mn_3O_4 added
20 c.c. . .	2 c.c. = 0·000304 grms. Mn_3O_4 .	0·0060	0·0033	1085·5	0·000649	0·000312	102·6
25 c.c. . .	2·5 c.c. = 0·00038 grms. Mn_3O_4. .	0·0088	0·0055	1447·5	0·000787	0·000359	94·5
25 c.c. . .	5 c.c. = 0·00076 grms. Mn_3O_4 .	...	...	...	0·001170	0·000750	98·7
25 c.c. . .	Do. .	...	...	...	0·001200	0·000780	102·6
25 c.c. . .	Do. .	...	...	...	0·001190	0·000770	101·3
50 c.c. . .	Do. .	...	...	...	0·001553	0·000713	93·8

It is seen that the gravimetric method yields figures about ten times higher than those obtained by the bismuthate method. The average figure for the recovery of added manganese as obtained by the gravimetric method is, however, too high—about 1266 per cent *i.e.*, more than twelve times the quantity added, while in the case of bismuthate it is only ninety-eight per cent of the quantity added.

The higher results obtained by the gravimetric method seem to be partly due to incomplete precipitation of iron during the removal of phosphates leading to a small fraction of basic iron acetate escaping into the filtrate, with the result that this is precipitated along with manganese by bromine. Evidence of the fact that the precipitate is liable to be contaminated with iron was obtained, by fusing the ignited precipitate of Mn_3O_4 from a 10 c.c. solution of manganese sulphate with potassium bisulphate, dissolving the fused mass in hot water and determining the iron in this solution by colorimetric method using potassium sulphocyanide to develop the colour. But the amount of iron present as impurity in Mn_3O_4 as determined by the gravimetric method is not enough to explain the considerable difference in the amount of Mn_3O_4 as determined by this and the bismuthate method Evidently the precipitate must be contaminated with other substances besides iron. In order to obtain some knowledge of these impurities in the ignited Mn_3O_4 and also to trace other factors responsible for the discrepancies in the results obtained by the two methods, manganese was estimated in another set of solutions in the presence of potassium aluminium sulphate, aluminium phosphate, calcium chloride and calcium phosphate and the results obtained are given below :—

TABLE IV

Showing loss of Mn_3O_4 during the process of removal of phosphates, due to incomplete precipitation and the presence of impurities in the precipitate

Description	Amount per 100 c.c. of the extract					Mn_3O_4 carried away with iron-aluminium phosphates and basic iron acetate (4)
	Bismuthate method (1)	Gravimetric method (2)	Composition of Mn_3O_4 obtained by the gravimetric estimation (column 2) (3)			
			Mn_3O_4 determined by the bismuthate method (3a)	Iron as Fe_2O_3 (3b)	Other impurities (by difference (3c)	
25 c.c. extract pot. alum. sulphate	0·00520	0·0144	0·00404	0·00100	0·00936	...
25 c.c. extract aluminium phosphate	0·00520	0·0312	0·00404	0·00463	0·02253	...
25 c.c. extract calcium chloride	0·00520	0·0120	0·00447	0·00018	0·00735	0·00037
25 c.c. extract calcium phosphate	0·00520	0·0120	0·00410	0·00125	0·00665	0·00061
25 c.c. extract . . .	0·00520	0·0080	0·00428	0·00034	0·00338	...
25 c.c. extract . . .	0·00520	0·0080	0·00441	0·00031	0·00328	...

As before the amount of manganese as found by the gravimetric method is much higher—varying from 150 to 600 per cent of that found by the bismuthate method and it would seem that the nature of the salts present in the solution, when bromine is added, exercises a considerable, though varying, influence on the amount of Mn_3O_4 finally obtained, and of the salts examined alumina seems to be the source of greatest error. In order further to test the purity of the ignited Mn_3O_4, the ignited precipitate obtained by the gravimetric method was fused with fused potassium bisulphate ; the fused mass after cooling was dissolved in hot water and made up to a definite volume. Manganese was determined in an aliquot portion by the sodium bismuthate method. The results obtained are given in column 3(*a*) of Table IV and approximate more closely to those obtained by the bismuthate method directly ; but they are now invariably lower. The cause of these low values seems to lie in the removal of a small amount of manganese with the iron-aluminium phosphates and basic iron acetate as is clear by the recovery of a fair amount of manganese from this precipitate in samples 3 and 4 (column 4). The sum of figures in columns 3 (*a*), and 4 is, however, still less than those in column 1, and these differences have been found to be due to the incomplete precipitation of manganese by bromine, the filtrate from which showed the presence of manganese by bismuthate method. Column 3 (*b*) of Table IV gives the amount of iron present as an impurity in the ignited Mn_3O_4 obtained by the gravimetric method. On further examination this solution was also found to contain traces of aluminium and phosphates. The inaccurate results obtained by the gravimetric method thus seem to be due to :—

(1) the removal of some manganese along with the iron-aluminium phosphate and basic iron acetate,

(2) the contamination of the final Mn_3O_4 precipitate with iron and aluminium phosphate, etc. and

(3) incomplete precipitation of manganese by bromine, whereby a small portion of manganese passes into the filtrate.

These results confirm Blum's observation [1912], that the composition of the precipitate in the gravimetric determination of manganese was not definite even under carefully regulated conditions. In addition to this main drawback, the gravimetric process is lengthy and tedious and requires almost continuous attention.

3. Estimation of manganese as influenced by variations in temperature and acidity

(*a*) *Vatiations of temperature.*—Blair [1904] has shown that the permanganic acid formed is not very stable in hot solutions and therefore recommends that the temperature of the solution before oxidation should be brought down to 15°C. In order to determine the range within which the temperature could be varied without affecting the accuracy of the results, determination of manganese was made in aliquot portions of the stock solution at different temperatures, with the results given below.

TABLE V

Estimation of manganese in plant material as influenced by the increase in temperature of the reacting solution

(Sample taken contained manganese equivalent to 0·0084 grm. of Mn_3O_4)

Temperature	Per cent Mn_3O_4 on oven-dried material.
10°C. . . .	0·0083
15°C. . . .	0·0083
20°C. . . .	0·0086
25°C. . . .	0·0083
30°C. . . .	0·0086
35°C. . . .	0·0061
40°C. . . .	0·0043

These figures indicate that the method is capable of giving accurate results at any temperature not exceeding 30°C.

(*b*) *Effect of variation in the concentration of the acid.*—Before proceeding with the oxidation of manganese with 4 *N* nitric acid, it is necessary to use a small amount of concentrated sulphuric acid in order to remove all traces of hydrochloric acid from the residue obtained by the evaporation of the hydrochloric acid extract of the plant ash. In order to remove all excess of sulphuric acid the contents must first be heated to dryness and then ignited. The process is not only lengthy but is also attended with the risk of loss of material due to decrepitation during ignition. In order therefore to determine the amount of sulphuric acid which could be allowed to remain behind after the complete removal of hydrochloric

acid, aliquot portions from the stock solution were taken and after the addition of 5 c.c. of sulphuric acid some of them were dried to the stage of production of white fumes of SO_3; while others were evaporated to complete dryness. The results given below indicate that this precaution is superfluous and the presence of sulphuric acid to the extent of 5 c.c. per 50 c.c. of 4 *N* nitric acid does not introduce any inaccuracy.

TABLE VI

Effect of the presence of concentrated sulphuric acid in varying amounts on the oxidation of manganese with 4 N HNO_3

(5 c.c. concentrated sulphuric acid were used in each case)

Volume of extract taken	Percentage amount of Mn_3O_4 determined	
	Sulphuric acid evaporated to dryness and ignited	Sulphuric acid evaporated to the stage when white fumes of SO_3 are produced
20 c. c.	0·0085	0·0084
25 c. c.	0·0084	0·0085
50 c. c.	0·0083	0·0084

The process of ignition to remove excess of sulphuric acid would thus seem to be quite unnecessary.

Table VII gives the amount of manganese found by taking different quantities of the same plant extract and it will be seen that the amounts of manganese per 100 grams of the oven-dried material calculated from all these determinations agree very closely with their mean value ($\chi^2 = 1 \cdot 619$, $P > 0 \cdot 9$).

TABLE VII

Volume of extract taken	Total Mn_3O_4 found	Per cent Mn_3O_4 on oven-dried material
20 c.c.	0·000342	0·0085
20 c.c.	0·000337	0·0084
25 c.c.	0·000428	0·0085
25 c.c.	0·000421	0·0084
25 c.c.	0·000413	0·0083
25 c.c.	0·000413	0·0083
25 c.c.	0·000428	0·0086
25 c.c.	0·000413	0·0083
50 c.c.	0·000428	0·0086
50 c.c.	0·000834	0·0083
	0·000841	0·0084

Mean = 0·00842 ±0·000111

The standard error of difference in the amount of maganese found by parallel estimations is so small that the bismuthate method for the determination of manganese can be relied upon to give excellent results under sufficiently wide range of conditions provided the precautions necessary for its estimation are properly observed.

In Table VIII are given the results of the estimation of manganese in the same set of six samples of fodders analysed by different workers using their own different methods. Samples No. 2 and 3 are duplicates of Nos. 6 and 5 respectively and were prepared from the same material, but this fact was not known to the analyst at the time of analyses. The set of figures given under gravimetric method (*a*) was obtained at the Chemical Research Laboratories of the Agricultural Chemist to Government Dacca, Bengal and that given under (*b*) at Mandalay, Burma. It will be seen that the gravimetric method not only gives higher figures than those obtained by the bismuthate and the periodate methods, but that in the hands of two different workers, the results obtained are liable to differ widely. The figures for the amount of manganese obtained with the bismuthate and the periodate method s how a fairly close agreement.

TABLE VIII

Relative values for the amount of manganese in different remples of fodders, as determined by the bismuthate, periodate and gravimetric methods

Bismuthate method	Periodate method	Gravimetric method	
		(a)	(b)
1. 0·009 . . .	0·008	0·003	0·407
2. 0·011 . . .	0·012	0·018	0·139
3. 0·006 . . .	0·008	0·009	0·282
4. 0·014 . . .	0·016	0·022	0·362
5. 0·007 . . .	0·008	0·027	0·075
6. 0·009 . . .	0·012	0·018	0·318

It is clear that the gravimetric method gives much higher figures, which are not identical in the hands of two different workers ; whereas the results obtained by the bismuthate and the periodate methods agree fairly closely.

SUMMARY

1. The gravimetric method for the estimation of manganese very often gives abnormally high values.

2. As judged by the concordance of results obtained with parallel estimations and the recovery of added manganese to the hydrochloric acid extracts of plant ash, the sodium bismuthate method for the volumetric determination of manganese in plant materials has been found to give excellent results.

3. In solutions containing different salts approximating in composition to the plant extract, the amount of manganese determined by this method agrees very closely with the amount known to be present.

4. For obtaining accurate results it is necessary (1) to use nitric acid free from all traces of nitrous fumes and (2) to remove every trace of hydrochloric acid before oxidation.

5. Temperature of the solution before and after oxidation must not exceed 30°C.

6. It is not necessary to remove sulphuric acid before carrying out the oxidation.

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UTILIZATION OF CERTAIN FORMS OF INORGANIC NITROGEN DURING THE DECOMPOSITION OF PLANT MATERIALS IN THE SOIL

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Although it is known that the adverse effect consequent on the decomposition of stubble can be largely remedied by liberal application of nitrates or ammonium salts, it is yet not clear as to which of these two forms is preferentially utilized by micro-organisms. A study in this direction has, however, been made by Richards and Shrikhande [1935] who found a preference for the ammoniacal over the nitric nitrogen in the early stages while working with plant products alone. A similar study in another direction has been made by Shive and co-workers [1933] by following the relative rates of absorption of ammoniacal and nitric nitrogen by plants grown in culture solutions. They observed greater ammonia absorption in the early stages than nitrate. The latter was absorbed to a maximum in the later stages. The reactions were largely controlled by the pH of the culture solution.

The phenomenon of the decomposition of plant residues in the soil in presence of different forms of inorganic nitrogen is more complicated than the study of decomposition of plant products alone. Such a study in the soil involves the consideration of at least four factors :—(*i*) absorption of ammonia by soil colloids, (*ii*) movement of the mineral forms of nitrogen in the soil depending upon its physical structure, (*iii*) nitrification of the decomposed organic matter initially present in the soil and losses of nitrogen through de-nitrification and (*iv*) assimilation of soluble mineral nitrogen by the micro-organisms in the soil to elaborate their tissue materials. The following investigation was, therefore, undertaken to study, under laboratory conditions, the relative rates of utilization of nitrogen in the ammoniacal and nitric forms by the micro-organisms concerned in the decomposition of plant tissues when both forms are almost equally available by following periodically the changes in ammoniacal, nitric and total nitrogen. The experiments were so arranged as to take into account the above-mentioned possible reactions going on in the soil. Two soils—one wet cultivated and the other dry cultivated—were used in the present study.

EXPERIMENTAL

The soils were air-dried and screened through a one-mm. sieve. Finely powdered *ragi* (*Eleucine coracana*) straw was added at the rate of three tons to

an acre. Hundred-gram lots of soil with 0·3 grm. each of straw passing through one-mm. mesh sieve, after thoroughly mixing, were placed in wide-mouthed bottles of 250 c.c. capacity. Ammonium sulphate and sodium nitrate were added in aqueous solution at the rate of one grm. of soluble nitrogen per 100 grms. of straw, approximately equivalent to the nitrogen factor [Hutchinson and Richards, 1921], *i.e.*, each bottle was supplied with 0·003 grm. (30 p. p. m.) of soluble nitrogen either wholly as ammonium sulphate or sodium nitrate, or 0·0015 grm. of each where an equivalent mixture of both was added. The moisture content was brought to about twenty per cent, the mixture well-stirred and the bottle plugged with cotton-wool. Loss of moisture was made up by adding water from time to time. Separate samples were incubated at 30°C. for the different periods of decomposition. Five sets of bottles containing five each for each soil were incubated. The analysis for each set was carried out at intervals of three, seven, fourteen, twenty-eight and fifty-six days, as was done in the case of pure plant products [Richards and Shrikhande, 1935]. Four sets with each soil contained straw while the fifth one was left untreated as control.

One bottle was removed at each stage and the following determinations were made on its contents :—

Ammoniacal nitrogen.—This was estimated in the potassium chloride extract at pH 1·0 as described by Ölsen [1929] by distillation with magnesia.

Nitric nitrogen.—This was determined on the residue after ammonia-nitrogen distillation by reduction with Devarda's alloy under alkaline conditions.

Total nitrogen.—This was estimated by the usual Kjeldahl method using sulphuric-salicyclic acid mixture [A. O. A. C., 1930].

Analytical figures for the soil nitrogen and the nitrogen content of straw are contained in Table I.

TABLE I

Analytical figures for the soil and straw (expressed as parts per million)

	Wet cultivated soil	Dry cultivated soil
Total nitrogen (soil)	174	720
NH_3-N	4	4
NO_3-N	2	9
N in the straw added	16	16
NO_3-N+NH_3-N added	30	30
∴Total N	220	766
Organic N	184	723

Table II deals with the figures for the different forms of nitrogen obtained experimentally and have been expressed as parts per million. The results for

both the soils have been included in the same table to facilitate comparison. Section (A) deals with wet cultivated soil alone ; (B) with soil+straw ; (C) with soil +straw+equal amounts of ammoniacal and nitric nitrogen ; (D) with soil+straw+ammoniacal nitrogen ; and (E) with soil+straw+nitric nitrogen ; the corresponding sections with dashes represent the figures for the dry cultivated soil.

TABLE II

Forms of nitrogen in parts per million (figures actually obtained)

Days	Wet cultivated soil				Dry cultivated soil			
	NH_3-N	NO_3-N	Total N	Organic N	NH_3-N	NO_3-N	Total N	Organic N
	Soil alone (A)				*Soil alone (A′)*			
3	4	..	189	185	13	15	721	693
7	11	3	180	166	9	15	711	687
14	10	8	164	146	12	16	700	672
28	12	14	165	139	11	22	716	683
56	10	16	166	140	10	30	718	678
	Soil+Straw (B)				*Soil+Straw (B′)*			
3	1	2	202	199	2	3	750	745
7	1	3	196	192	1	2	749	746
14	1	2	19	190	2	2	744	740
28	1	2	..	180	1	1	..	739
56	1	2	198	195	1	1	751	749
	Soil+Straw+NH_3-N+NO_3-N (C)				*Soil+Straw+NH_3-N+NO_3-N (C′)*			
3	17	25	..	233	24	33	724	667
7	14	18	215	183	12	33	744	699
14	12	6	215	197	13	26	755	716
28	6	6	215	203	10	31	755	714
56	11	..	202	177	9	21	760	730
	Soil+Straw+NH_3-N (D)				*Soil+Straw+NH_3-N (D′)*			
3	20	11	244	213	37	21	757	699
7	17	8	228	203	17	14	751	720
14	12	3	215	200	10	16	750	724
28	6	2	216	208	9	20	754	725
56	15	2	205	188	10	18	..	742
	Soil+Straw+NO_3-N (E)				*Soil+Straw+NO_3-N (E′)*			
3	8	36	241	197	13	41	757	703
7	9	30	191	151	14	26	745	705
14	11	18	202	173	13	19	..	737
28	11	9	213	193	19	21	760	720
56	12	16	216	187	9	31	748	708

Considering the case of soil alone (sections A and A'), the ammoniacal nitrogen remains practically stationary for both the types of soil, whereas there is a gradual increase in the amount of nitric nitrogen with the period of incubation, suggesting the presence of decomposing organic matter in the soil. Evidence to this effect was also obtained while treating the soil with hydrogen peroxide during the course of mechanical analysis. The difference between the amount of nitrate on the 3rd and 56th days is practically of the same order (about 15 p. p. m.) in both the soils. When straw is incorporated into the soil (Section B and B') there is considerable fall in the values for both the ammonia and nitrate. These lower values are no doubt due to the assimilation of both the forms by the micro-organisms during the decomposition of the straw. The utilisation of ammoniacal nitrogen is usually complete, partly due to its absorption by the soil colloids and partly to its assimilation by the micro-organisms for their structural development, thus allowing little or none for losses due to volatilisation. But where nitric nitrogen is involved, it is necessary to consider that the gradual fall in nitrate may be due not only to assimilation by the micro-organisms but also to loss as elementary nitrogen, although it would appear from an examination of the value for total nitrogen that this latter is negligible.

Surveying the results as they appear in Table II, without accounting for the control, the following observations can be made :—

Section (C and C').—In the wet cultivated soil when ammonia and nitrate have been added in equal amounts, the quantity of ammonia is practically constant, whereas there is a heavy drop in the nitric nitrogen as the period of incubation increases. With dry cultivated soil under identical conditions the reverse is the case.

Section (D and D').—The addition of ammonia nitrogen to the soil and straw mixtures appears to result in a rapid fall of the ammonia content of the dry cultivated soil with time, whereas with the wet cultivated one the change is not so marked. The changes in the nitrate content are the reverse of the above. It is possible that in the dry cultivated soil the decrease in the ammonia content may be due to assimilation by micro-organisms since the figure for total nitrogen remains practically constant. The greater preference of ammonia in the dry cultivated soil may also be accounted for by considering the relative rates of movement of ammonia and nitrate in the soil. It is known that nitrate moves down faster than ammonia in a light soil, thus removing it from the reach of the organisms. They have, therefore, to seek recourse to what is readily available. The movement of the two forms of nitrogen is dealt with separately. The constant values for the nitrate in the dry cultivated soil may be more apparent than real because of the nitrification of the decomposed organic matter initially present in the soil.

Section (E and E').—The addition of nitrate to the soil treated with straw does not bring about any marked variation in the nitrate content of the dry

cultivated soil, but in the wet cultivated one a rapid fall in the nitrate content is observed. A comparison with the figure for total nitrogen in the latter case shows that this fall in the nitrate content may possibly be due to loss by de-nitrification.

By subtracting the figures for ammoniacal and nitric nitrogen from the total nitrogen present in a particular sample, an estimate of the organic nitrogen present in it can be made. It will be observed in all the three treatments mentioned above, that both the total and organic nitrogen are almost of the same order with the dry cultivated soil as those initially present. With the wet cultivated soil, however, it is difficult to state with the available data whether nitrogen is not lost by denitrification and probably also due to volatilisation as ammonia, with the result that there is usually a fall in all the cases of the organic nitrogen.

The above observations are more clearly brought out from the results included in Table III where allowance has been made for values obtained with the control experiments.

TABLE III

Figures expressed in parts per million after accounting for the figures in the control experiments

Days	Wet cultivated soil		Dry cultivated soil	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N
	Effect of straw on the soil NH_3 and NO_3 nitrogen			
	(*A*)		(*A'*)	
3	3	..	11	12
7	10	..	8	13
14	9	6	10	14
28	11	12	10	21
56	9	14	9	29
	Soil+Straw+NH_3-N+NO_3-N			
	(*B*)		(*B'*)	
3	16	23	22	30
7	13	15	11	31
14	11	4	11	24
28	5	4	9	30
56	10	..	8	20

TABLE III—*contd.*

Days	Wet cultivated soil		Dry cultivated soil	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N
	Soil+Straw+NH_3-N			
	(*C*)		(*C'*)	
3	19	9	35	18
7	16	5	16	12
14	11	1	8	14
28	5	..	8	19
56	14	..	9	17
	Soil+Straw+NO_3-N			
	(*D*)		(*D'*)	
3	7	34	11	38
7	8	27	13	24
14	10	16	11	17
28	10	7	18	20
56	11	14	8	30

Section (*A and A'*).—The ammonia is practically the same as in the soil alone (Table II, Sections A and A'), whereas there is a difference between the nitrate content of the soil alone and when straw is introduced into it. There is, however a greater variation in the nitric nitrogen in wet cultivated than in the dry cultivated soil. The almost steady figures for nitrate in the dry cultivated soil may be ascribed to the greater amount of nitrification for which the conditions are more favourable due to its coarse texture and consequently greater aeration.

Section (*B and B'*).—The absorption of nitrate in the wet-cultivated soil is double that of ammonia between the 3rd and the 7th day and nearly five times as much between the 7th and the 14th day. In the dry cultivated soil nitrate is practically constant throughout the period of incubation while ammonia is heavily used up. These observations show that the organisms have a preference for ammonia in the dry cultivated soil whereas in the wet cultivated soil the low recovery of nitrate might either mean a preferential utilization by the organisms or a loss of nitrate due to de-nitrification. The latter explanation seems to be more probable because of the low values obtained for the total nitrogen wherever nitrate has been added. Further work is needed to establish either of the two possibilities.

Section (*C and C'*).—Here more than thirty per cent of the total ammonia has been used up in the first three days in the wet cultivated soil, the absorption getting slower as the period increases. In the dry-cultivated soil however, ammonia

absorption is practically negligible in the first three days and maximum in the next ten days. This apparently contradictory result for the wet cultivated soil can be accounted for by considering the abundance of ammonia which is more than double of that present in section (B and B'). The relatively higher figure for nitrate in the dry cultivated soil may be due to the conversion of some of the ammonia into nitrate during diffusion as pointed out by Cook [1916] and also due to nitrification.

Section (D and D').—Ammonia is almost constant and nitrate is utilised in both the soils, the maximum absorption being in the earlier stages in the dry cultivated soil and in the wet cultivated in the later stages. Such a difference may be accounted for by the relative rates of movement of the two forms of nitrogen in the two different soils.

Relative movements of ammonia and nitrate nitrogen in the soil

That there is a difference in the relative rate of downward movement of the two forms of nitrogen depending upon the physical structure of the soil has been pointed out by several workers.

Karpizov [1904] in investigating the absorptive capacity of different soil layers, found it at a maximum in the surface layers decreasing directly with the humus and zeolites towards the sub-soil. The degree of absorption varied with the mechanical composition and the hygroscopic moisture content of the soil. Sabanin [1908] confirmed the observations of Karpizov. Zhottsinski [1908] working with Russian soils noted a relationship between the mechanical composition and ammonia absorption. Cook [1916] while studying the retention and distribution of ammonium sulphate found that ammonia moves down quicker to lower layers in the light but in the heavy soils most of the ammonia is held in the top surface of three or four inches. The experience of these workers indicates that the movement of ammonium sulphate in the soil is slow and actual rate varies with the nature of the soil. Nitrogen in the nitric form is more easily diffused than nitrogen in any other form as has been pointed out by several workers.

To study on a small scale whether these observations hold good for the experiments described in this paper both the types of soils were filled in to a height of nine inches in cylinders of 9-in. height and 2½-inch diameter made of paraffined paper. The cylinders were made to stand in glass dishes of about the same diameter. To give a greater stability for the cylinders they were placed in a wooden chest with frame for each. The soil which weighed about 600 grms. was brought to a moisture content of twenty per cent and then poured into the cylinder. Nitrogen in the form of solution of ammonium sulphate and sodium nitrate to the extent of 0·05 grm. was poured on the soil surface. Cylinders thus made facilitate cutting the soil columns at a particular depth with any metal or glass plate preventing any mixing of the soil with other layers.

The usual depth of surface soil is reckoned as top 9 in. and hence the particular height of the soil column in this experiment. The cylinders were cut at depths

of 3 in. and 6 in. from the top and the amount of ammonia and nitrate estimated at intervals of three, seven and thirty days. The results obtained are included in Table IV. They agree with the observations of foregoing workers. The movement of ammonia is greater in the dry soil than in the wet one. There is also an increase in the nitrate in different layers in the red soil due partly to the conversion of ammonia into nitrate but mostly to the nitrification of the decomposed organic matter initially present. In the paddy soil, however, nitric nitrogen is practically constant. The greater movement of nitrate in the red than in the paddy soil can clearly be seen by comparing the drop from 0·051 grm. to 0·043 grm. in the former with 0·045 grm. to 0·043 grm. in the latter between the 3rd and the 7th day. The comparatively high value of 0·044 grm. nitrate in the red soil on the 30th day must be due to the nitrification of the organic matter. This fact is also borne out by the increasing amounts of nitrate in the different layers and also by the progressive nitrate production during the incubation of soil alone.

TABLE IV

NH_3 and NO_3 nitrogen content of different soil layers during the movement of the two forms of nitrogen in the soil

(*Figures in grm. actually obtained in the experiment*)

Days	Top 3-in. layer		Middle 3-in. layer		Bottom 3-in. layer	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
	Dry cultivated soil+0·05 grm. NH_3-N					
3 . .	0·050	0·003	0·005	0·004	0·003	0·002
7 . .	0·043	0·003	0·003	0·003	0·003	0·003
30 . .	0·040	0·008	0·002	0·001	0·001	0 003
	Wet cultivated soil+0·05 grm. NH_3-N					
3 . .	0·046	0·001	0·001	..	0·008	..
7 . .	0·044	0·001	0·001	..	..	..
30 . .	0·044	0·001	0·001	..	0·001	..
	Dry cultivated soil+0·05 grm. NO_3-N					
3 . .	0·003	0·051	0·003	0·003	0·003	0·003
7 . .	0· 02	0·043	0·002	0·003	0·00	0·003
30 . .	0·002	0·044	0·001	0·004	0·001	0·004
	Wet cultivated soil+0·05 grm. NO_3-N					
3 . .	..	0·045	..	0·003	..	0·001
7 . .	..	0·043	..	0·005	..	..
30 . .	0·001	0·040	..	0·007	..	..

Discussion

As a result of these studies a few observations can be made with regard to the selection and application of a nitrogenous fertilizer to the soil. The salt used as fertilizer splits up almost immediately into ions. The cations are absorbed by the soil colloids and the anions liberated. Ammonia from ammonium sulphate is absorbed by the soil colloids, and may later become available for the structural development of the micro-organisms. In the case of sodium nitrate, however, the sodium, after splitting will be absorbed by the soil complex letting the nitric nitrogen free which becomes available to the organisms and the plant. The results of the present experiments have shown that in the dry cultivated soil ammoniacal fertilizer in conjunction with organic manures might prove useful in the conservation of soil organic matters. But as stated previously it is difficult to say with the available evidence as to what may happen when nitrates are added to the soil especially in the wet cultivated one. Another factor which plays an important part in fertilising practice is the movement of soluble forms of nitrogen in the soil. This movement, of course, depends upon the nature of the soil as evidenced by several workers. In the wet cultivated soil ammonia is absorbed by the colloids leaving little for the downward movement whereas the nitrate is free to move about. In the dry cultivated soil such a movement of both the ions is facilitated by the deficiency of soil colloids as also by greater pore space on account of the coarser nature of the soil praticles. The nature of the soil should, therefore, be a guide in the application of a soluble nitrogenous fertilizer. In the heavy soil nitrate ought to be a better fertilizer or a supplement when organic matter is added or a crop sown. It may be applied either as nitrate of chalk or ammonia rather than as sodium nitrate which might produce an adverse effect on physical the structure of the soil. Moreover it will have less chance of leaching here than in the light soil. In the light soil, ammonia should be better adapted as it will be more easily available than in the heavy. As nitrate moves faster than ammonia in the light soil it would be more economic to apply a dressing of ammonia than nitrate which, on account of its greater downward movement and solubility, may be washed down and removed from the zone of activity of the plant and the micro-organisms.

Summary

(1) Incorporation of straw with soil depresses the amount of ammoniacal and nitric nitrogen present in the soil.

(2) When straw with an almost equal dressing of ammonium sulphate and sodium nitrate are introduced into the soil, there is a definite preference by the micro-organisms concerned in the decomposition for ammoniacal nitrogen in the dry cultivated soil over nitric nitrogen whereas reverse may be the case in the wet cultivated soil. Further work is needed to establish the preferential utilisation in the wet—cultivated soil.

(3) Nitrate appears to move down faster than ammonia in both the soils. Compared amongst themselves, ammonia goes down quicker in the dry cultivated than in the wet cultivated soil. The rate of movement is rather slow.

(4) Practical applications of the results obtained have been discussed.

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ON THE NATURE OF NITRIFICATION IN SOIL

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Nitrification in soils has been generally accepted to be a bacterial process. Recently Dhar, Bhattacharya and Biswas [1933] have suggested that nitrification in tropical soils is more photo-chemical than bacterial in nature. Rossi [1933] also believes that rapid nitrification in soil can take place by purely physico-chemical process.

Sarkaria and Fazal-ud-Din [1933] tested the photo-catalytic power of some Punjab soils, and found that the soils showed only a weak catalytic action. Similar results were obtained by Fraps and Sterges [1935] with some American soils.

In view of the great importance of nitrification in soils, experiments were undertaken to find out whether photo-nitrification or bacterial nitrification predominated in the Punjab soil.

METHODS OF ANALYSIS

Nitrous nitrogen. By Griess Illosvay reagent.

Nitric nitrogen. By Phenol-disulphonic acid.

Chlorides, whenever present in excess, were completely removed by precipitation with silver sulphate before treatment with the reagent.

EXPERIMENTAL

Freshly air-dried soil was passed through one mm. sieve and mixed with ammonium phosphate [*$(NH_4)_2$ HPO_4] at the rate of fifty-seven mgs. of nitrogen per 100 grams of soil. The treated soil was put in nine glass jars at the rate of 1800 grams per jar. The jars were divided into three sets of three each. In each set, soil of one jar was mixed with nine grms. of mercuric chloride to eliminate all living organisms, the second was covered with a metallic lid to prevent access of light and the third was left uncovered.

In the first set, the soil was kept air-dry, in the second, at thirteen per cent moisture content, and in the third, at thirty-nine per cent moisture content. All the jars were wrapped with black paper on the outside and exposed to the sun. Loss in moisture was made up from time to time. The experiment was started on 28th March. Samples were analysed after 65 days and 100 days. The results are given in Table I.

* According to Dhar and others [1933] ammonium phosphate was found to be more rapidly nitrified than any other compounds of ammonia.

TABLE I

Nitrification of ammonium phosphate in soil

(Mgs. of nitrogen per 100 grms. of soil)

Treatment		65 days			100 days		
		Nitrous N	Nitric N	Percentage oxidised	Nitrous N	Nitric N	Percentage oxidised
Air-dry	Sterilized and exposed.	Trace	4·5	0·2	Trace	7·7	5·7
	Unexposed .	,,	4·8	0·7	,,	6·0	2·8
	Exposed .	,,	4·8	0·7	,,	7·5	5·4
13 per cent moisture.	Sterilized and exposed	Trace	4·6	0·4	Trace	6·3	3·3
	Unexposed .	,,	11·6	12·6	,,	12·0	13·3
	Exposed .	,,	7·2	4·9	,,	9·5	8·9
39 per cent moisture.	Sterilized and exposed	Trace	4·0	...	Trace	4·4	0·0
	Unexposed .	,,	4·0	...	,,	4·4	...
	Exposed .	,,	3·2	...	,,	1·5	...

Initial { Ammoniacal nitrogen . . . . . 57·0 mgs.
Nitrous nitrogen . . . . . Trace
Nitric nitrogen . . . . . 4·4 mgs.

It will be seen from the above that :—

(1) under air-dry condition of the soil, nitrification was insignificant either by biological or photo-chemical means during first sixty-five days, while on exposure to 100 days, 5·8 per cent of the added nitrogen was converted into nitrate in presence of light as compared with 2·8 per cent in the absence of light.

(2) At optimum moisture content no nitrification took place by photo-chemical process but 12·6 per cent nitrified by the biological process during first sixty-five days, and in 100 days the photo-chemical nitrification was 3·3 per cent as compared to 13·3 per cent by the biological means.

(3) Under saturated moisture contents no photo-nitrification took place even after 100 days' exposure, and nitrogen was reduced rather than oxidised by the biological process.

Very low nitrification by the biological process, during the experiment, was found to be due to the temperature of the soil, which was very high (above 48°C.) and inhibitory after the first fortnight of the experiment.

Fazal-ud-Din [1935] noticed that the catalytic action of the soil was different for different nitrogenous compounds. In order to form a correct estimate of photo-nitrification as compared to biological nitrification, it was thought necessary to try the nitrification of a number of compounds by both the processes.

Fresh, air-dried soil was passed through one mm. sieve and different nitrogenous compounds were added to supply forty mgs. nitrogen per 100 grms. of soil. In the first series, the moisture was made up to thirteen per cent by saturated mercuric chloride solution, while in the second, it was made up by distilled water. The soils so prepared were taken in 200 grm. portions in duplicate conical flasks of 500 c.c. capacity. The flasks were plugged with cotton wool and in the first series they were exposed to the sun, while in the second series, they were wrapped in black paper and kept side by side.

As the experiment was intended to run for three months, the loss of moisture had to be made up frequently, therefore sterilization of the soil by treatment with mercuric chloride was preferred to steam sterilization to avoid a chance contamination with nitrifying bacteria and consequent error.

The experiment was started on 12th January and terminated on 12th April, when the soils were analysed for nitrous and nitric nitrogen. The results given in Table II indicated that the different nitrogenous compounds were nitrified to varying degrees (from 36·0 to 64·7 per cent) by the biological process, while under the same conditions no nitrification took place by photo-chemical means.

TABLE II

Nitrification of different nitrogenous compounds in soil

(Mgs. of nitrogen per 100 grms. of soil)

Nitrification of	Photo-nitrification		Bacterial nitrification		
	Nitrous N	Nitric N	Nitrous N	Nitric N	Percentage oxidised
Ammonium chloride .	Trace	3·1	0·2	17	36·0
Ammonium sulphate .	,,	3·1	0·3	21	46·2
Ammonium acetate .	,,	2·5	0·2	28·5	64·7
Ammonium oxalate .	,,	2·5	0·2	26·5	59·7
Glycocoll . . .	,,	2·3	0·4	19	41·5
Acetamide . . .	,,	2·5	0·3	19	41·2
Creatinine . . .	,,	2·5	0·2	20	43·5
Urea	,,	2·6	0·2	23	51·0
Uric acid . . .	,,	2·9	0·2	20	43·5

Initial { Nitrous nitrogen . . . . . Trace
Nitric nitrogen . . . . . 2·8 mgs.

The absence of photo-nitrification in the above series suggested the possibility of the inhibitory action on the process by mercuric chloride treatment. Although mercuric chloride did not show any poisoning effect on the catalytic action of the soil in the previous experiments, nevertheless, it was of interest to investigate its effect on the action of a powerful catalyst.

One hundred c.c. of $\underline{N}/2$ ammonium chloride solution and one gram of zinc oxide were taken in each of the four beakers of 400 c.c. capacity. Two of these beakers were treated with half a gram of mercuric chloride. These were exposed to the sun and washed air was slowly bubbled through each at approximately the same rate. Nitrous nitrogen was estimated after six hours. The results given below showed that mercuric chloride did not poison the catalyst.

Treatment	Mgs. of nitrous nitrogen per 100 c.c. of the solution	
Without mercuric chloride	0·26 0·22	0·24
With mercuric chloride	0·22 0·19	0·20

In the case of mercuric chloride treated solutions, oxidation was slightly reduced. This was partly due to the suspension becoming slightly greyish and partly due to the reduced surface of the catalyst because of the conglomeration of the fine particals of zinc oxide. However, the effect was only slight.

Experiments were undertaken to find out how much increase in photo-nitrification was obtained by incorporation of a small amount of a powerful catalyst with soil. For this purpose two per cent zinc oxide was mixed with some soil. Ammonium sulphate was added at the rate of forty mgs. of nitrogen per 100 grams of soil and the mositure content made up to thirteen per cent. Two hundred and eighty grams of the prepared mixture was equally distributed into four flasks of 300 c.c. capacity. Four control flasks were similarly set up without zinc oxide. The flasks were plugged with cotton wool and sterilized at 120°C. for one hour on two consecutive days. The plugs were covered with paper. The flasks were exposed to the sun and analysed after a month for nitrous and nitric nitrogen. There was no difference in the nitrate contents of the flasks. There was, however, some difference in the nitrite contents as shown below :—

Treatment	Mgs. of nitrous nitrogen per 100 grms. of soil	
Soil + zinc oxide	0·44 0·46 0·44 0·44	0·44
Soil alone	0·22 0·24 0·20 0·13	0·20
Difference		0·24

This showed that the catalytic power of the soil could be increased by the addition of a powerful catalyst but not to any great extent.

Dhar and co-workers found that ammonium phosphate was almost completely nitrified photo-chemically in three months' time. Compared to that the

photo-nitrification of ammonium phosphate in Lyallpur soils was very small i,e., 5·7 per cent. The soils of the Punjab were found to be weak photo-catalysts [Sarkaria and Fazal-ud-Din, 1933], therefore nitrification in these soils appeared to be mainly biological in nature. In summer the rate of photo-nitrification was very small while in winter no photo-nitrification took place.

As regards the high nitrate content of the surface soils during summer months which has been attributed by Dhar and others as being due to the active photo-oxidation, it might be explained by the rise of nitrates from lower depths due to the increased rate of evaporation during summer months. Bacterial nitrification during summer is inhibited on the surface on account of the high temperature, low moisture contents, and sterilizing action of the rays. But nitrification goes on at lower depths where conditions are more favourable. Thus it would be apparent that the process of nitrification is mainly a biological one in the tropical soils of the Punjab, and the small amount of oxidation by photo-chemical process which can take place only in summer, is of very small importance.

After studying the oxidation of various nitrogenous compounds in soil, some experiments were undertaken to study the comparative rates of nitrification in solution by the two processes.

Fifty c.c. of dilute (1 : 3) Omeliansky solution less ammonium sulphate with one per cent calcium carbonate were put in conical flasks of 300 c.c. capacity. Various nitrogenous compounds were separately added in sets of four flasks to supply five mgs. of nitrogen per flask and sterilized. Volatile compounds were added after sterilization. Each flask was inoculated with one gram of soil, wrapped in black paper and kept over roof.

Another series of seventy-two flasks was divided into two sets. In one set, one gram of zinc oxide, and in the other five grams of soil were added in each flask. The same nitrogen compounds as used in the first series were added at the same rate to the four flasks of each set and the volume was made up to fifty c.c. with distilled water, sterilized and kept over roof. Both the series were started together in February. The contents of the flasks were analysed after two, three and four weeks. The results are given in Table III. The results of oxidation in presence of soil catalyst have been omitted as the oxidation was negligibly small.

Photo-oxidation with zinc oxide as a catalyst was more than bacterial oxidation during the first two weeks. During the third week, the oxidation by bacteria increased and finally in the fourth week, it was forty-seven to ninety-seven per cent. Maximum photo-oxidation after four weeks was only nineteen per cent.

It was found that the rate of multiplication of nitrifying bacteria was very slow in Omeliansky solution and therefore the rate of oxidation of nitrogen is slow in the beginning, later on when sufficiently large number of organisms are present the rate is very swift and even a few hours suffice for the appreciable oxidation of the nitrogen added as ammonium sulphate.

TABLE III

Nitrification in solution

(Mgs. of nitrogen per 100 c.c. of solution)

Nitrification of	Bacterial						Photo (zinc oxide as catalyst)					
	Two weeks		Three weeks		Four weeks		Two weeks		Three weeks		Four weeks	
	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N
Am. hydroxide	0·1	0·1	0·4	0·2	6·8	0·2	Trace	0·8	Trace	1·2	Trace	1·6
Am. sulphate	0·4	0·1	7·8	0·6	9·1	0·3	,,	1·0	,,	1·2	,,	1·9
Am. carbonate	0·4	0·1	4·9	0·3	5·5	0·3	,,	0·6	,,	1·5	,,	1·4
Am. acetate	0·3	0·3	3·2	0·2	6·2	0·2	,,	0·3	,,	..	,,	0·8
Am. oxalate	0·1	0·1	0·3	0·1	6·5	0·2	,,	1·1	,,	1·9	,,	1·4
Glycocoll .	0·1	0·1	0·1	0·1	4·5	0·2	,,	0·5	,,	0·6	,,	1·1
Acetamide .	0·1	0·1	0·7	0·1	6·8	0·2	,,	0·3	,,	0·5	,,	0·9
Creatinine .	0·2	0·1	2·5	0·2	4·5	0·4	0·1	0·6	,,	0·6	,,	0·8
Urea .	0·3	0·1	1·7	0·2	0·1	9·6	Trace	0·6	,,	0·5	,,	1·1
Initial { Nitrous nitrogen . .	trace											
Initial { Nitric nitrogen . .	0·3											

In a number of 300 c.c. flasks, twenty c.c. of dilute Omeliansky solution was inoculated with six c.c. of actively nitrifying Omeliansky solution and incubated at 35°C. It was found that about one milligram of ammoniacal nitrogen was oxidised in six hours.

TABLE IV

Rate of nitrification in Omeliansky solution

Incubation period (hours)	Mgs. of nitrous nitrogen per 100 c.c. of solution
Initial	2·75
12	3·89
18	4·86
24	5·83
36	7·78
42	9·31

This showed that in an actively nitrifying solution the rate of oxidation by bacteria is many times more than the oxidation by the sun. If however, the medium is not favourable for the growth of nitrifying bacteria, then bacterial nitrification becomes negligible in comparison to oxidation by solar radiations as has been shown elsewhere [Fazal-ud-Din, 1935].

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STUDIES IN THE GERMINATION OF CROP SEEDS IN THE FIELD

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I. INTRODUCTION

The importance of good stand in a crop can hardly be over-emphasized. Too thick or too thin a stand always results in a low yield and consequently makes farming less remunerative. The need for a good stand is equally important in field experiments. Unless the stand of a crop in differently treated plots is uniform and comparable, the effect of various treatments under trial will be obscured. Anything which is likely to assist in obtaining a good stand is equally important for the farmer and for the agricultural experimenter engaged in field experiments.

The stand of a crop depends upon the quantity of seed sown per unit area, its germination capacity and the survival of the seedlings after their emergence from the soil. Seed rate can be varied at will. Germination of seeds when they are viable depends upon (1) the physical nature of soil, (2) its preparation, (3) moisture content and (4) the depth at which seeds are sown. Of all these factors, No. 4 can be measured and controlled with minimum effort, but still very little definite information with regard to the range of depth from which the seeds of common crops can germinate in a particular soil is available. Consequently some work was done in this direction the results of which are presented in this paper.

The depth at which seeds are sown not only affects germination but it may affect the after-growth of the seedlings and consequently influence the yield. Martin [1930] says that potatoes planted half-inch deep gave 16·5 bushels per acre more than deeper plantings. White [1934] found that sweet potatoes covered with one inch sandy soil yielded 2,260 lbs. of primes per acre more than those covered three inches deep. Johnsen and Nielsen [1934] sowed spring cereals at nine depths from zero to twenty cm., spaced at intervals of 2·5 cm, and found that development of seedlings and older plants from seeds planted at depths greater than five cm. was delayed and they showed less vigour and poorer colour. According to Rees [1931] the best depth for sowing sainfoin is 3/4 inch to 1/4 inch.

In order to find out the optimum depths for sowing common crops at Lyallpur their seeds were sown at different depths. This work was primarily undertaken for our own guidance in securing better stand in experimental plots, but it is now felt that this information may be of use to other workers and consequently it is brought to their notice.

II. Method and material

Seeds used in this study were hand-picked. The soil in which these tests were made is loam and is representative of the soils of the district. It contains clay 8·36 per cent, silt 14·63 per cent, fine sand 62·72 per cent and sand 14·3 per cent. It was irrigated in the usual way with canal water and seed-bed was prepared by means of spade. Moisture content of the seed-bed was determined. It generally varied from 11·61 to 12·03 per cent. Seeds were sown by means of sowing tubes devised by the authors [Labh Singh, Nathuram and Nek Alam, 1934] at ten known depths varying from one half to five inches, the interval between consecutive depths being half an inch. Fifty seeds of each crop were sown at each depth in rows 2½ inches apart, the distance from seed to seed in the row being also 2½ inches. There were six such replications in randomised blocks, thus the total number of seeds of each crop sown being 300 at each depth.

Germination tests of each crop were repeated several times in the year but results of only those tests are given here which were made during the main sowing season of each crop. Seeds were sown in the evening. Observations to record germination were commenced twenty-seven hours after sowing and repeated regularly every three hours, *viz.*, 9 P.M., 12 P.M., 3 A.M. and 6 A.M., etc. A seed was considered to have germinated when the seedling had emerged above the ground. At each observation the seedlings that had come above the ground were counted and pulled out so that counting at the next observation may be facilitated.

III. Germination at various depths

Altogether fourteen summer and eight winter crops were dealt with, their local, English and Botanical names are given below :—

Season of growth	Serial No.	Local name	English name	Botanical name
Summer	1	*Rawan*	Cowpea	*Vigna catjang* Walp.
,,	2	*Mung*	Green gram	*Phaseolus radiatus* L.
,,	3	*Moth*	Moth	*Phaseolus aconitifolius* L.
,,	4	*Mash*	Black gram	*Phaseolus mungo* L.
,,	5	*Sani*	San (hemp)	*Crotalaria juncea*
,,	6	*Gwara*	Fieldvetch or Gwara	*Cyamopsis psoralioides* DC.
,,	7	*Makki*	Maize	*Zea Mays* L.
,,	8	*Chari*	Great millet	*Andropogon Sorghum* L.
,,	9	*Sudan Ghas*	Sudan Grass	*Sorghum sodanc uris* L.
	10	*Bajra*	Bulrush millet	*Pennisetum typhoideun* Rich.

Season of growth	Serial No	Local name	English name	Botanical name
Summer	11	*Swank* . .	Swank . .	*Panicum colonum* L.
,,	12	*Til* . .	Sesamum . .	*Sesamum indicum* D C.
,,	13	American *kapas*	4-F American .	*Gossypium hirsutum* L.
,,	14	*Desi kapas* .	15 Mollisoni .	*Gossypium indicum* Lam.
Winter	15	*Chana* . .	Gram . .	*Cicer arietinum* L.
,,	16	*Kanak* . .	Wheat . .	*Triticum vulgare* Host.
,,	17	*Jaon* . .	Barley . .	*Hordeum vulgare* L.
,,	18	*Jai* or *Jawi* .	Oats . .	*Avena sativa* and *A. sterilis* L.
,,	19	*Shalgum* .	Turnips . .	*Brassica rapa* L.
,,	20	*Taramira* .	Rocket . .	*Eruca sativa* L.
,,	21	*Toria* . .	Indian rape .	*Brassica napus* L.
,,	22	*Sarson* . .	Indian colza .	*Brassica campestris* Prain

As already stated germination counts were made after every three hours. The detailed results are given in Appendix I. They are represented below graphically. Fig. 1 relates to fourteen summer crops and Fig. 2 represents eight winter crops :—

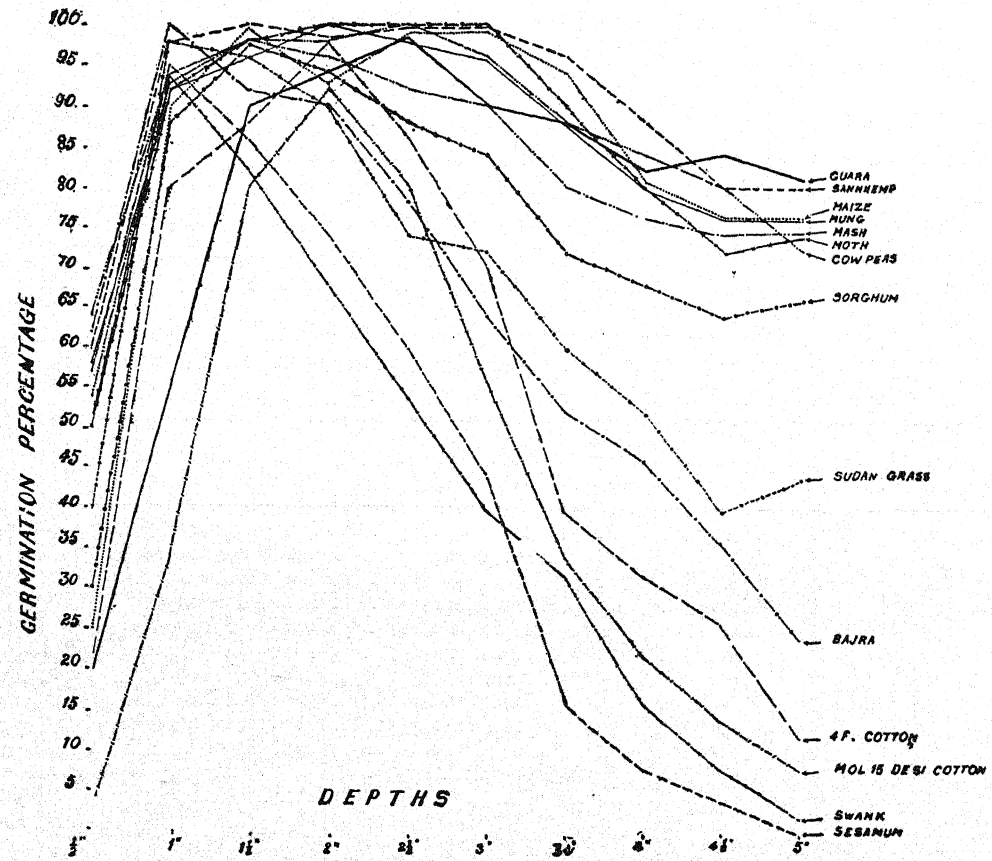

Fig. 1. Showing the germination percentage of fourteen summer crops at different depths.

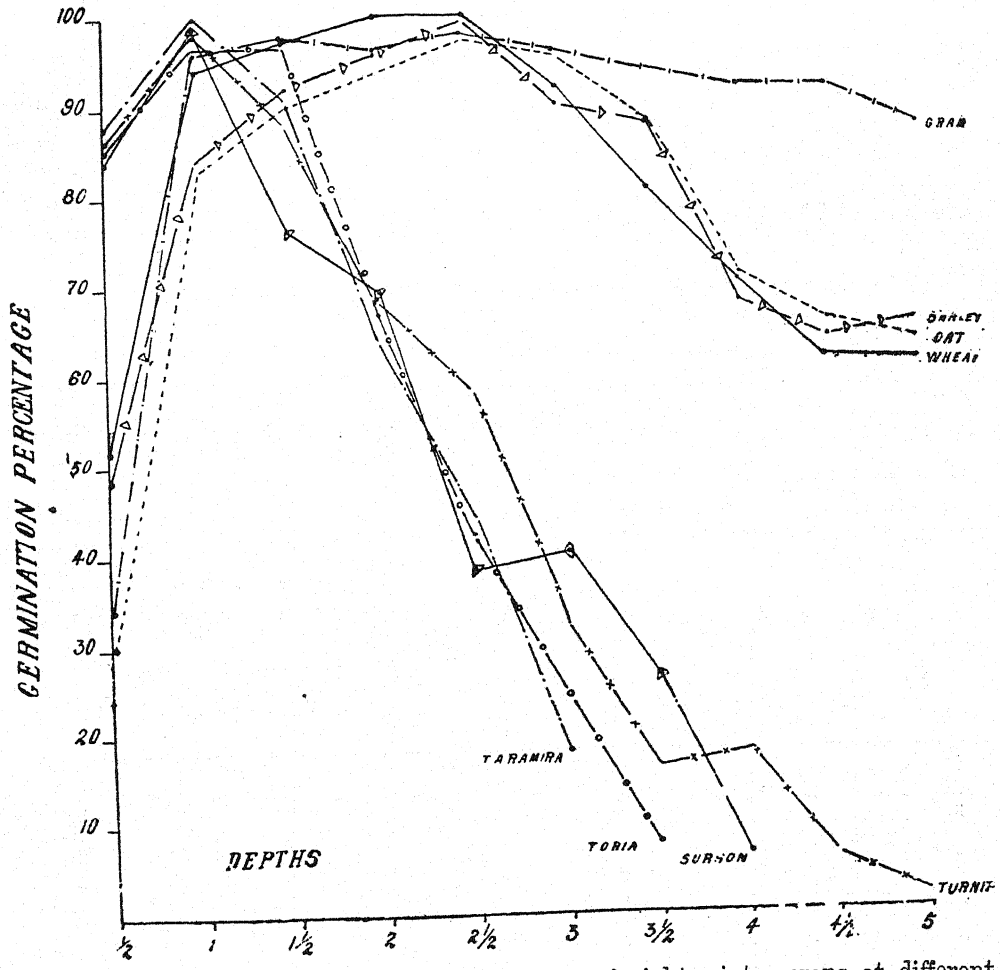

Fig. 2 Showing the germination percentage of eight winter crops at different depths.

The whole data have been examined statistically. The general effect of depths on germination is significant in all the crops. The standard errors of the mean differences have been worked out by analysis of variance and are given in Appendix I referred to above.

It would be seen from Appendix I and Figs. 1 and 2 that each crop has given good germination at more than one depth. For instance, gram has germinated almost equally well at depths 1 to 4½ inches (92-98 per cent). The question now arises as to the optimum depth for sowing this crop. To answer this question the range of depth is fixed by taking germination at higher and lower depths which did not differ significantly from the maximum germination percentage. (Critical difference was taken to be twice the standard error of mean difference). The middle depth of this range where the number of depths is odd, and the first of the two middle-most depths where the number is even, has been taken as the optimum depth of the crop. For instance, eight depths from 1 to 4½ (1, 1½, 2, 2½, 3, 3½, 4, 4½) in the case of gram did not differ significantly from each other. Hence 2½ inches have been taken as optimum depth. Proceeding in this manner the optimum range and optimum depth for each crop has been determined and is given in the following table :—

Serial No.	Crop	Optimum depth in inches	Optimum range of depths in inches	At optimum depth, time of start, finish and period of germination in hours		
				Start	Finish	Period
1	Cowpeas	2	1-3	48	69	21
2	*Mung*	2	1-3	54	75	21
3	*Moth*	2	1-3	54	84	30
4	*Mash*	2	1-3	60	84	24
5	San-hemp	2	1-3½	42	63	21
6	*Gwara*	2½	1½-3½	51	93	42
7	Maize	2½	2-3½	72	108	36
8	*Chari*	1½	1-2½	54	87	33
9	S. Grass	1½	1-2	48	84	36
10	*Bajra*	1½	1-2	42	84	42
11	*Swank*	1	1-1½	60	93	33
12	*Til*	1	1-1½	48	87	39
13	4-F American	2	1½-2½	78	129	51
14	Mollisoni 15 *Desi*	1½	1-2	75	126	51
15	Gram	2½	1-4½	102	123	21
16	Wheat	2	1-3	114	147	33
17	Barley	2½	1½-3½	126	168	42
18	Oats	2½	1½-3½	135	177	42
19	Turnips	1	½-1½	72	99	27
20	*Taramira*	1	½-1½	57	90	33
21	*Toria*	1	½-1½	72	111	39
22	*Sarson*	1	½-1½	72	96	24

In 2nd column of the above table is given the optimum depth and in the 3rd column is given the range of depths between which germinaion was not significantly different from the maximum germination percentage. In order to get good germination, seed should under ordinary conditions be sown at its optimum depth but in those soils which lose moisture rapidly from the top especially during hot and dry weather or when seed-bed is not well prepared it should be sown between the optimum depth and the highest limit of its optimum range. Optimum depth and optimum range would vary according to the character of soil. They would be shallow in stiff and deep in loose soils. Further it would be observed that range in some crops is wide and even an indifferent sowing in their case would give good germination as in case of gram, san-hemp, pulses, etc. They are not so sensitive to depth. The range of some crops such as *swank*, sesamum, cotton, rapes, turnips, etc., is very narrow and to get a good germination of these crops is not always an easy task. They are more sensitive to depth. More care is, therefore, required with regard to depth while sowing these crops.

IV. Time taken for the start and completion of germination by different crops

As stated in the beginning observations were started twenty-seven hours after sowing and were repeated after every three hours. It was observed that some crops started to germinate earlier than others although they were sown at the same time and other conditions were kept as uniform as possible. The time when germination was completed was also noted in each case. The difference in the time of start and the time of completion of germination is the period during which a crop germinated. This interval is called the period of germination. Detailed data in this connection are given in Appendix II at the end.

A reference to Appendix II would indicate that in general the shallower the depth the quicker is the start of germination. Maize, gram and *gwara* took, however, longer to start germination at ½ inch than at one inch. Other main features of Appendix II are :—

(1) Almost all seeds at half an inch took longer time to complete germination than at one inch, probably because soil moisture is less in the first half inch of soil than in the second half inch (at one inch depth).

(2) On the whole, period of germination is smaller at depths lower than the optimum depth within the optimum range.

V. Germination at various hours of the day

As already stated germination counts were taken after every three hours throughout the day and night, *viz.*, 3 A.M., 6 A.M., 9 A.M., etc. In this way seeds that germinated between 3 A.M. and 6 A.M. were recorded at 6 A.M. and those germinated between 6 A.M. and 9 A.M. were noted at 9 A.M. and so on. It was observed that the rate of germination at all depths and more or less for all crops is rapid

during certain hours whereas at others it was very slow. Maximum germination took place between 3 A.M. and 6 A.M. It was next followed by the interval between midnight and 3 A.M. During noon hours germination proceeded very slowly. Least germination took place during the hours from 6 to 9 P.M. The rate of germination during the different hours of the day for all crops at their optimum depth is given in Appendix III.

The average germination percentage of all crops that took place at different hours of the day is given below :—

Period of the day (hours) . .	0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24
Germination percentage . .	18·1	36·4	16·7	5·4	4·2	7·1	2·4	7·4

The figures of germination percentage given in the 2nd row above are the result of sowing 6,600 seeds of the twenty-two crops under observation.

In crops as pulses and san-hemp germination, once it has started, is finished more or less within first twenty-four hours. In cotton it takes more than forty-eight hours. In the case of crops which take more than twenty-four hours for the completion of germination, the rate of germination during the various hours of the day during the second twenty-four hours was in the same order as in the first twenty-four hours.

In all the tests the data relating to which have so far been discussed seeds were sown at 6 P.M. In order to see whether seeds sown at different hours of the day would behave in a similar manner, Mollisoni 15 Desi and 4-F American cottons were sown at their optimum depths on the 24th May, 1933, at 6 P.M., 7 A.M. (next morning) and 12 noon. The land for all these sowings was prepared at one and the same time. Observations were recorded after every hour in this case. The mean maximum and minimum temperature and relative humidity during the days of the experiment were 108·1°F. and 76·1°F. and 36·8 per cent respectively. The detailed data recorded after every one hour are given in Appendix IV. To make it comparable with the previous data, recorded after every three hours, data recorded after every one hour was grouped into periods of three hours, *viz.*, 3-6, 6-9, etc., etc., and the results of the grouping are given below :—

Variety	Time of sowing	No. of hours after sowing when germination started	Hours of the day when germination started	Percentage germination during the hours from								Total
				0 to 3	3 to 6	6 to 9	9 to 12	12 to 15	15 to 18	18 to 21	21 to 24	
Mollisoni 15 *Desi*	6 P.M. . .	60	6 A.M. .	10	16	34	14	2	2	...	12	90
	7 A.M. (next day)	66	1 A.M. .	18	34	30	8	...	...	...	4	94
	12 NOON . .	61	1 A.M. .	26	38	24	...	...	...	...	4	92
4-F American .	6 P.M. . .	62	8 A.M. .	16	24	34	4	...	...	2	14	94
	7 A.M. (next day)	67	2 A.M. .	18	32	34	6	...	...	...	8	98
	12 NOON . .	61	1 A.M. .	18	38	32	4	...	...	4	4	100

These figures in general confirm the previous observations, *viz.*, greater percentage of seeds germinated during the cool hours after mid-night irrespective of the time of sowing. After 9 A.M. germination declines steadily. There is very little germination in the afternoon and evening.

Another interesting point which would be observed from the foregoing table is that *desi* cotton sown at 6 P.M. started to germinate sixty hours after sowing whereas cotton sown at 7 A.M. took sixty-six hours and cotton sown at 12 noon sixty-one hours. 4-F American has also behaved in the same manner. The question arises what is this difference due to ? Apart from other things seed would germinate after it has been in the soil for a certain period and if this period ends when the hours to follow are favourable for germination it begins to germinate, but if at the end of this period the following hours are not favourable for germination, it seems as if the crop waits for favourable time to come. *Desi* cotton sown at 6 P.M. completed sixty hours after sowing at 6 A.M., a time which is favourable for germination. So it began to germinate. On the other hand, cotton sown at 7 A.M., completed its sixty hours at 7 P.M. when time was not favourable for germination. It had, therefore, commenced to germinate at 1 A.M. and thus took sixty-six hours to start germination. Noon sowing commenced germinating after sixty-one hours at 1 A.M., when time was favourable for germination. 4-F American cotton has also behaved in the same manner.

Should land be ready in the afternoon, at present the practice is to sow summer crops in the evening even if men and bullocks have to be over-strained because it is supposed that evening sowings give better germination than sowings made next morning. The experiments described above, however, clearly show that evening sowings have no special advantage over sowings done next morning. After the preparation of land the question whether summer crops should be sown on the same evening or next morning should solely be decided according to the facilities available and not by the necessity of sowing at a particular hour of the day.

Summary

(1) A good stand of a crop is important for the practical farmer as well as the agricultural experimenter. The depth at which seed is sown not only affects the germination percentage of seed and the survival of young seedlings but also the ultimate stand of a crop. Seeds of twenty-two common crops were sown at the depths from half to five inches, interval between the successive depths being half inch, *viz.*, $\frac{1}{2}$, 1, $1\frac{1}{2}$, etc., and germination counts were recorded after every three hours.

(2) Optimum depth in loam soil for turnips, *taramira*, *toria*, *sarson*, sesamum and *swank* is one inch ; for sorghum, Sudan grass, *bajra*, and *desi* cotton $1\frac{1}{2}$ inches for cowpeas, *mung*, *moth*, *mash*, sann-hemp, 4-F American cotton and wheat two inches and for *gwara*, maize, gram, barley and oats $2\frac{1}{2}$ inches.

(3) Irrespective of the depth and the time at which seeds were sown the rate of germination was very rapid from midnight to 9 A.M., while it was very low from noon to midnight.

(4) Practical farmers in the Punjab believe that sowing of summer crops in the evening gives better germination than sowing next morning. The results obtained in this test do not confirm this belief.

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APPENDIX I

Percentage of seeds that germinated from different depths

No.	Crop †	DEPTHS IN INCHES										S. E. of the M. D.*
		½″	1″	1½″	2″	2½″	3″	3½″	4″	4½″	5″	
1	Cowpeas	64	94	98	98	100	96	88	84	80	72	3·4
2	*Mung*	58	92	96	100	98	96	88	80	76	76	3·6
3	*Moth*	50	92	98	100	100	100	90	80	72	74	4·1
4	*Mash*	54	92	98	96	92	90	80	76	74	74	3·9
5	Sann-hemp	60	98	100	98	100	100	96	88	80	80	2·4
6	*Gwara*	20	56	90	94	98	90	88	82	84	80	4·2
7	Maize	4	34	80	92	100	100	94	80	76	76	4·2
8	Sorghum	30	88	98	94	88	84	72	68	64	66	4·6
9	Sudan Grass	40	98	96	90	74	72	60	52	40	44	3·9
10	*Bajra*	60	100	92	90	78	64	52	46	36	24	4·1
11	*Swank*	52	92	82	68	54	40	32	16	8	2	5·4
12	Sesamum	58	94	86	74	60	44	16	8	4	...	4·7
13	4-F. American cotton	20	80	88	98	86	70	40	32	26	12	7·1
14	Mollisoni 15 Desi	22	90	100	92	80	56	34	22	14	8	6·8
15	Gram	34	96	98	96	98	96	94	92	92	88	2·7
16	Wheat	52	94	98	100	100	92	80	70	62	62	4·2
17	Barley	48	84	92	96	100	90	88	68	64	66	5·6
18	Oats	30	84	90	94	98	96	88	70	66	64	4·9
19	Turnips	86	98	88	68	58	32	16	18	6	2	6·3
20	Taramira	88	100	90	64	44	18	...	...	...	...	6·9
21	*Toria*	86	96	96	66	42	24	8	...	...	...	4·9
22	*Sarson*	84	98	86	68	38	40	26	6	...	...	7·4

* This has been calculated on the basis of the S. D. per plot calculated by Fisher's analysis of variance.

† Botanical names of crops are given in Table I.

APPENDIX

The number of hours after which germination started and

DEPTH

No	Crops	½			1			1½			2		
		S	F	P	S	F	P	S	F	P	S	F	P
1	Cowpeas . .	39	84	45	39	60	21	48	66	18	48	69	21
2	*Mung* . . .	42	87	45	48	66	18	54	72	18	54	75	21
3	*Moth* . . .	33	69	36	42	63	21	54	84	30	54	84	30
4	*Mash* . . .	39	87	48	39	69	30	54	81	27	60	84	24
5	Sann-hemp . .	30	63	33	33	48	15	39	60	21	42	63	21
6	*Gwara* . . .	54	99	45	48	87	39	48	87	39	48	90	42
7	Maize . . .	87	114	45	69	114	45	72	105	33	72	108	36
8	Sorghum . .	45	108	63	54	87	33	54	87	33	60	105	45
9	Sudan Grass .	45	108	63	45	81	36	48	84	36	48	90	42
10	*Bajra* . . .	36	84	48	39	84	45	42	84	42	48	87	39
11	Swank . .	60	132	72	60	93	33	60	108	48	60	108	48
12	Sesamum . .	54	72	18	48	87	39	57	108	51	57	135	78
13	4-F American cotton.	75	132	57	78	132	54	78	132	54	78	129	51
14	Mollisoni 15 Desi .	69	129	60	72	126	54	75	126	51	75	129	54
15	Gram . . .	108	171	63	96	117	21	99	120	21	99	120	21
16	Wheat . .	102	141	39	105	135	30	105	138	33	114	147	33
17	Barley . .	108	183	75	120	168	48	120	168	48	120	168	48
18	Oats	126	186	60	129	186	57	132	180	48	132	186	54
19	Turnips . .	60	102	42	72	99	27	93	135	42	96	147	51
20	*Taramira* . .	54	105	51	57	90	33	63	111	48	81	132	51
21	*Toria* . . .	60	108	48	72	111	39	84	120	39	84	156	72
22	*Sarson* . .	63	111	39	72	96	24	72	111	39	84	132	48

S—Denotes the No. of hours after sowing when germination started.

F—Denotes the No. of hours after sowing when germination was completed.

P—Denotes the No. of hours during which germination took place.

II

finished and period of germination at different depths

IN INCHES

2½			3			3½			4			4½			5		
S	F	P	S	F	P	S	F	P	S	F	P	S	F	P	S	F	P
51	90	39	54	102	48	57	111	54	57	111	54	63	111	48	63	114	51
54	90	36	57	90	33	60	99	39	60	114	54	63	126	63	66	126	60
54	96	42	57	102	45	57	108	51	66	126	60	69	132	63	69	138	69
60	87	27	66	93	27	69	108	39	69	111	42	69	138	69	78	138	60
45	75	30	45	87	42	48	96	48	48	96	48	51	120	69	51	126	75
51	93	42	54	114	60	60	120	60	63	123	60	63	132	69	78	150	72
72	108	36	75	114	39	78	114	36	78	114	36	78	117	39	78	117	39
60	114	45	60	114	54	60	114	54	60	114	54	63	114	51	63	120	57
54	99	54	57	114	57	57	126	69	60	135	75	60	135	75	69	144	75
48	90	42	48	108	60	54	114	60	63	132	69	63	144	81	81	153	72
60	111	51	66	114	48	72	114	42	72	114	42	84	114	30	84	114	30
72	132	60	81	138	57	84	138	54	84	156	72	120	156	36	...	...	...
81	156	75	81	156	75	81	165	84	84	204	120	90	213	123	132	249	117
78	153	75	81	159	78	81	162	81	87	207	120	87	222	135	99	222	123
102	123	21	108	138	30	114	159	45	114	165	51	120	168	48	126	171	45
114	144	30	117	147	30	123	156	33	129	177	48	132	183	51	141	189	48
126	168	42	126	171	45	132	177	45	144	192	48	150	201	51	150	204	54
135	177	42	135	183	48	150	201	51	156	207	51	159	207	48	162	207	45
101	168	67	144	171	27	144	171	27	150	180	30	153	183	30	168	192	24
84	144	60	87	156	69	...	...	...	...	...	...	...	...	...	...	...	...
84	159	75	102	159	57	105	159	54	...	...	...	...	...	...	...	...	...
87	138	51	90	156	66	108	156	48	132	156	24	...	...	...	...	...	...

Appendix III

Percentage of seeds germinated during the different hours of the day from optimum depth for each crop

No.	Crop	Optimum depth in inches	0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24	Total
1	Cowpeas . .	2	24	24	12	2	2	14	..	20	98
2	*Mung* . .	2	34	30	8	..	6	2	4	16	100
3	*Moth* . . .	2	24	38	20	8	2	2	2	4	100
4	*Mash* . . .	2	10	22	30	6	12	10	4	2	96
5	Sann-hemp . .	2	18	26	4	4	4	2	14	26	98
6	*Gwara* . . .	2½	28	26	16	6	..	6	6	10	98
7	Maize . . .	2½	22	34	18	8	6	..	2	10	100
8	Sorghum . .	1½	24	44	20	4	..	2	..	4	98
9	Sudan Grass . .	1½	8	38	18	2	8	16	6	2	98
10	*Bajra* . . .	1½	4	36	8	2	6	22	2	12	92
11	*Swank* . . .	1	2	52	8	8	4	16	..	2	92
12	Sesamum . .	1	8	42	26	10	4	2	2	..	94
13	4-F American cotton	2	22	32	24	6	4	4	..	6	98
14	Mollisoni 15 *desi* .	1½	26	34	22	4	2	2	2	8	100

15	Gram . . .	2½	8	38	8	8	6	14	4	12	98
16	Wheat . . .	2	12	26	20	8	6	16	4	8	100
17	Barley . . .	2½	34	28	14	6	10	..	..	8	100
18	Oats . . .	2½	20	48	20	8	..	..	..	2	98
19	Turnips . .	1	18	52	18	2	4	4	..	..	98
20	*Taramira* . .	1	14	38	26	10	4	8	..	..	100
21	*Toria* . . .	1	28	36	16	2	2	4	..	8	96
22	*Sarson* . . .	1	10	56	12	4	..	10	2	4	98
	Average . .		18·1	36·4	16·7	5·4	4·2	7·1	2·4	7·4	97·7

APPENDIX

Germination percentage of cotton (Desi and American) in

Variety	Time of sowing	No. of hrs. after which germination started	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Mollisoni 15 *desi*	6 P.M. .	60	...	...	...	...	...	2	4	4	8	8	2	...	...	2	...	...	...
	7 A.M. .	66	6	2	6	4	10	6	14	8	6	6	2	...	...	...	...	...	...
	12 NOON	61	2	6	8	14	8	8	10	8	2	...	...	...	...	...	...	...	...
4-F American	6 P.M. .	61	...	...	...	...	...	...	...	2	6	2	...	...	...	...	...	...	...
	7 A.M. .	67	...	4	8	4	8	6	10	8	14	4	2	...	...	...	...	...	...
	12 NOON	61	4	4	6	4	14	16	8	10	12	4	...	...	...	...	...	...	...

IV

each hour of the day when sown 2 inches deep at different hours of the day

18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9	10	11	12	Afterwards upto 144 hours	Total
2	...	...	...	2	8	2	2	4	4	2	2	6	8	4	6	4	...	...	4	92
...	...	...	...	...	2	2	2	...	2	4	4	4	2	...	...	...	...	...	2	94
...	...	...	...	...	2	2	4	2	4	...	2	2	...	2	...	...	...	...	6	92
...	...	2	...	4	4	6	6	4	6	4	6	14	...	12	26	2	...	...	6	94
...	...	...	...	2	4	2	2	4	...	2	4	2	...	2	...	...	...	...	6	98
...	2	2	...	...	2	2	...	2	2	...	...	4	...	...	2	...	...	...	...	100

A PHOMA DISEASE OF ASPARAGUS

BY

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(Received for publication on 19th February 1936)

(With Plate XLIII)

Asparagus is extensively grown in some parts of Europe and America from where canned asparagus are exported. In India its cultivation is limited to certain parts of the country and in addition to its use as a vegetable, the berries are often employed medicinally by the *hakims*.

Symptoms

In 1935 asparagus plants at Pusa were found to be affected by a fungus disease. The first symptom is the discoloration of the tissue of the stem into light brownish areas which become dark brown as the disease advances. The affected parts become shrivelled but do not collapse (Plate XLIII, A). The tissues at the centre of the spots become ashy white and later show small dark bodies. These are seen on microscopic examination to be the pycnidia of a fungus. The pycnidia break through the epidermis which cracks longitudinally. The growth of lateral branches is frequently checked (Plate XLIII, B). The disease appears on all parts of the plants except the berries but is principally confined to the main stem. In advanced cases the needles become yellow, and the plants may become completely or partially defoliated, and finally wither. The pith of the affected stem becomes ashy white and spongy, owing to the invasion of the fungus hyphae.

Pathogenicity

Cultures of the fungus were obtained by planting sterilised bits of affected tissues on Quaker-oats agar slants and from these pure cultures were re-isolated.

Several successful inoculation experiments were made at Pusa during the rains and autumn on unwounded and wounded asparagus stems and pathogenicity of the fungus was thus proved. The method employed was to place fragments of culture or of naturally infected tissue in contact with the healthy stems—either with or without wounding—and to keep moist by binding round with damp cotton wool. As might be expected infection took place more rapidly on wounded than on unwounded plants. The influence of climatic conditions on the incidence of the disease was indicated by observation that both natural and artificial infection took place more readily during the monsoon than during the following months. The fungus was re-isolated from the inoculated parts and compared with the parent culture and was found to be identical.

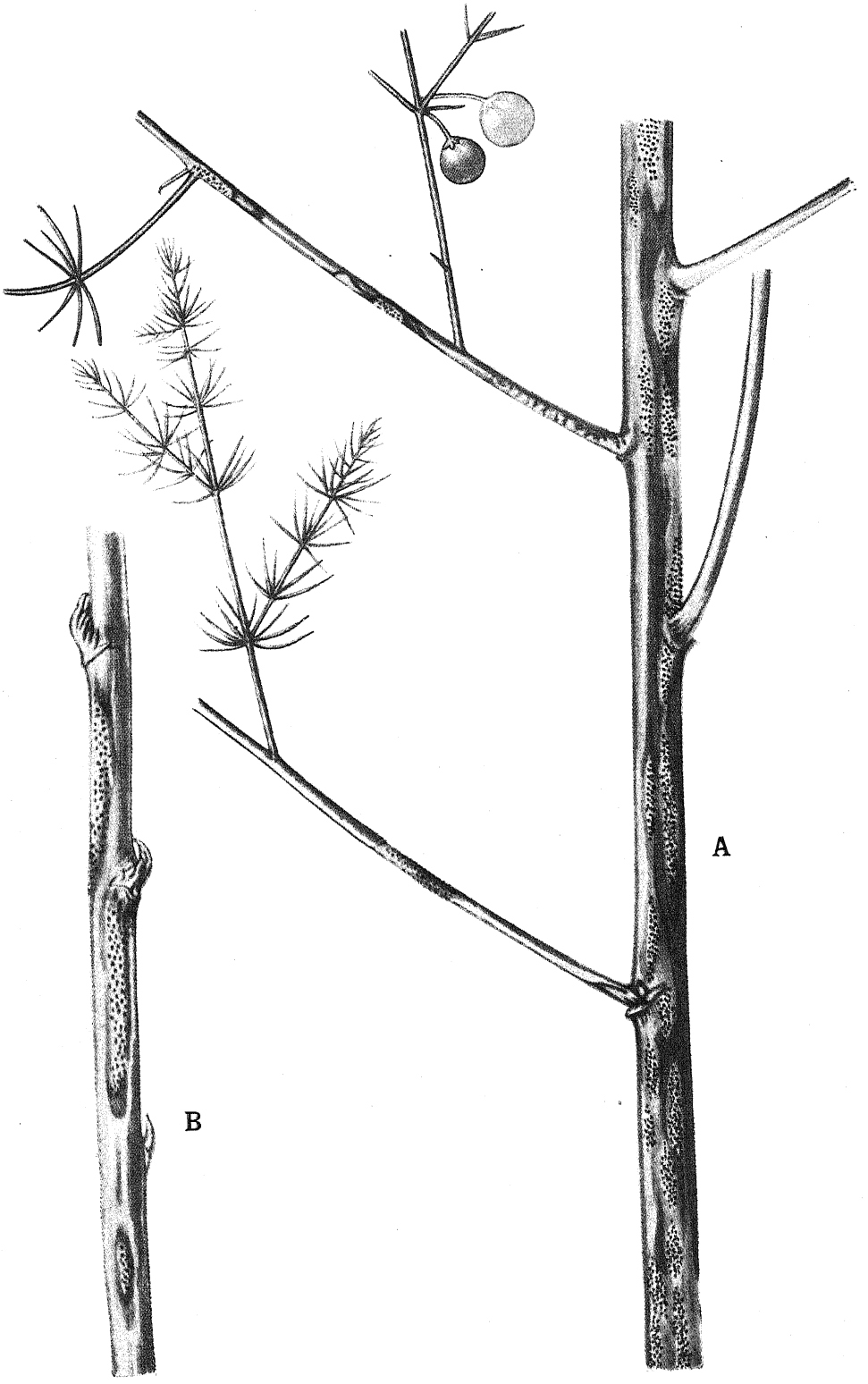

FIG. (A) Asparagus stem showing the attack of the fungus ($\times 1\frac{1}{5}$)

FIG. (B) Young asparagus stem showing withered lateral buds ($\times 1\frac{1}{3}$)

Morphology of the fungus in culture

On rich nutrient media like Quaker-oats agar the fungus grows profusely. Growth is at first confined to the substratum, and when the fungus reaches the edge of the slant aerial mycelium is formed. The mycelium is at first hyaline and then turns light brown, and the hyphae vary in breadth. As the culture grows old vesicles are formed in the hyphae. Light brown pycnidia are formed at the margin of the agar slant, but fail to mature. On sterilised fragments of asparagus stems, however, pycnidia with numerous pycnospores are readily formed. On Dox's agar, Brown's agar, and Hopkin's agar the growth is poor, and pycnidia are rarely formed.

Microscopic characters

Sections were made of the infected stems of asparagus and examined under a microscope. The mycelium is composed of septate hyaline threads which ramify in all parts of the stem. It is both intra and inter-cellular. Comparatively little mycelium is found in the sclerenchymatous tissues, but in the bundles and the pith it is found abundantly. The pycnidia are usually borne under the epidermis and when they are mature they break through it. The conidia are oblong, rod or spindle-shaped, hyaline and one-celled. They measure $5 \cdot 1$–$10 \cdot 2 \times 1 \cdot 7$–$3 \cdot 4 \mu$. The conidiophores are not distinctly visible.

Systematic position

The fungus was first described by Fuckel [1869] as the spermogonial form of *Diaporthe asparagi* Fuckel, and Saccardo [1884] who named it *Phoma asparagi* Sacc. was also inclined to regard it as a pycnidial stage of *Diaporthe*. Bubak [1906] stated that in *P. asparagi* he found A and B forms of spores and all variations between them and re-named the species *Phomopsis asparagi* (Sacc.) Bubak. He described the A spores as oblong or spindle-shaped and gave the extreme variations of the size as $5 \cdot 5$–$9 \times 2 \cdot 2$–5μ. The range of 200 spores of the Pusa fungus is $5 \cdot 1$–$10 \cdot 2 \times 1 \cdot 7$–$3 \cdot 4 \mu$ which agrees with the A form of spores of Bubak. As the B form of spores are not found in the Indian material it cannot be *Phomopsis*. It may also be stated that Diedicke [1912] who had made a special study of the genus *Phomopsis* did not accept *Phoma asparagi* as a *Phomopsis* but listed it under *Phoma*. Grove [1935] lists the species both as *Phoma* or *Phomopsis*. Teng [1932] has recorded *Phoma asparagi* from China. There are three distinct species of *Phoma* known on asparagus. The present species agrees with *Phoma media* Ell. et Ev. in the size of spores but differs from it in not having distinct conidiophores. It also differs from *Phoma lanceolata* (C. et E.) Sacc. in having much smaller spores. It closely agrees with *Phoma asparagi* Sacc. in the shape and size of spores, structure of pycnidia, and therefore it is considered to be *Phoma asparagi* Sacc. This appears to be the first record of the fungus in India.

ACKNOWLEDGMENT

Thanks are due to Mr. E. W. Mason of the Imperial Mycological Institute, for the help rendered in the identification of the fungus, and for references to the literature.

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GUMMOSIS OF CITRUS IN BOMBAY

BY

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(With Plates XLIV-XLVI and two text-figures)

INTRODUCTION

Gummosis, one of the commonest and most destructive maladies of citrus, has an extensive geographical distribution. In the Bombay Presidency it was first recorded on the pomelo, **Citrus grandis* Osbeck, in 1910, although it is probable that the disease had existed in the citrus orchards for a long time. The disease, however, did not attract much attention until 1923 when serious outbreaks were reported on sweet orange locally known as *mosambi*, a horticultural variety of *Citrus sinensis* Osbeck, from important centres of citrus cultivation. In a few years the disease has been widely distributed, and there is now hardly a locality in the Bombay-Deccan free from it.

Fawcett [1923] published the results of his experiments in 1923, but since he came to the conclusion that pathological effects associated with gum formation were produced by a number of independent organisms, it was considered necessary to undertake an investigation into the nature of the disease as it occurs in Bombay, the conditions influencing its development, and the method of prevention and treatment. Unfortunately, there have been many interruptions in the progress of the work, and it has thus not been possible to publish these results at an earlier date. This paper embodies the results obtained during the period from 1925 to 1932, inclusive.

NOTE—The writers acknowledge the assistance rendered by Mr. M. K. Desai, formerly Ag. Assistant Mycologist, in field experiments.

* The names of species and varieties of Citrus used in this paper are according to W. T. Swingle (" Citrus " in Bailey's Standard Cyclopaedia of Horticulture, Vol. 2, pp. 270-785, New York, 1914) and G. S. Cheema and S. S. Bhat (" A Study of the Citrus Varieties of the Bombay Presidency " in *Current Science*, Vol. 2, pp. 298-304 [1934.)

A valuable review of literature dealing with gummosis and brown rot of citrus has been published by Fawcett [1923, 1 ; 1923, 2.]

Symptoms

In Bombay gummosis produces typical symptoms in *mosambi* and affects the trunk. In the field it is characterised by the copious exubation of a resinous gummy substance and the cracking of the bark for considerable distances upwards from the bud union (Plate XLIV, figs.1 and 2) ; in an advanced case of attack there may be extensive shedding of the bark exposing the wood below (Plate XLIV, fig. 3). This is followed by the yellowing and drooping of leaves, and the affected trees look as if suffering from the effects of a drought (Plate XLIV, fig. 4).

In the early stage of the disease, if the bark in the invaded region is scraped with a knife, the inner surface shows brown discoloration which gradually fades at the margins of the invaded zone into the normal colour of the sound tissue (Plate XLV, fig. 1). It is beyond the invaded region that the fungus is most actively growing. The bark remains firm at first, but ultimately shrinks and cracks longitudinally.

In time the invaded region extends considerable distances upwards from the bud union but relatively smaller distances sideways. Invariably there is a rapid upward extension from the bud union, and the bark may also crack longitudinally 50 to 60 cm. or more in length. The most serious aspect of the disease is in the lateral extension of the invaded region, and when more than two-thirds of a trunk is girdled, treatment becomes difficult and the tree eventually dies (Plate XLIV, figs. 3 and 4).

In the area surrounding an advanced region, a watery substance is produced underneath the bark, and as the gum accumulates in large quantities near the cambium, the bark is separated from the wood below, thus forming gum pockets. The pressure ultimately is so great that the bark cracks longitudinally and masses of watery gum come to the surface where they soon dry by loss of water. There is often such a copious exudation of gum that masses of it run down the trunk and accumulate at the base in the dry season.

In badly affected trees the dead bark dries and sloughs off. The wood thus exposed, if unprotected, is attacked by the wood-rotting fungi and decays.

In wet or damp weather the fruit is also attacked. During the rains the fungus produces the active motile spores or zoospores, which are the principal agents in the spread of infection. These zoospores are splashed from the soil to fruits on branches almost touching the ground, and germinate by hyphae which enter the rind or underlying tissue. After a few days an oily spot appears on the surface (Plate XLV, fig. 2 (*a*)), which rapidly enlarges in extent until the whole fruit is involved. The affected fruits fall to the ground, and the fungus soon comes out on the surface as small white patches of mycelium.

FIG. 1. A *mosambi* tree affected with gummosis showing cracking of bark.

FIG. 2. Same as Fig. 1 and accompanied by copious exudation of gum.

FIG. 3. An advanced case of gummosis showing extensive shedding of bark, resulting in the death of the tree.

FIG. 4. A severely affected tree showing drooping of leaves. The tree ultimately succumbed to the disease.

FIG. 1. An affected tree in which diseased bark was removed with a knife. Note an area of brown discoloration in the centre surrounded by healthy tissue.

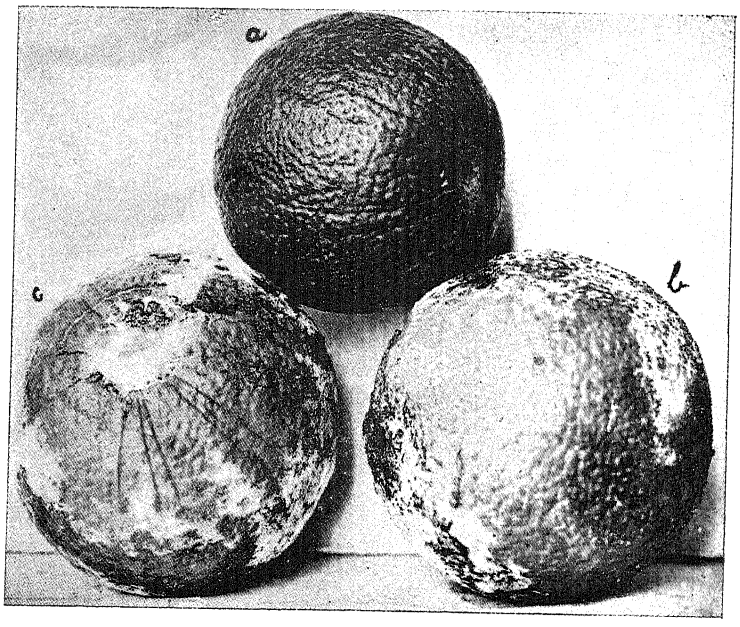
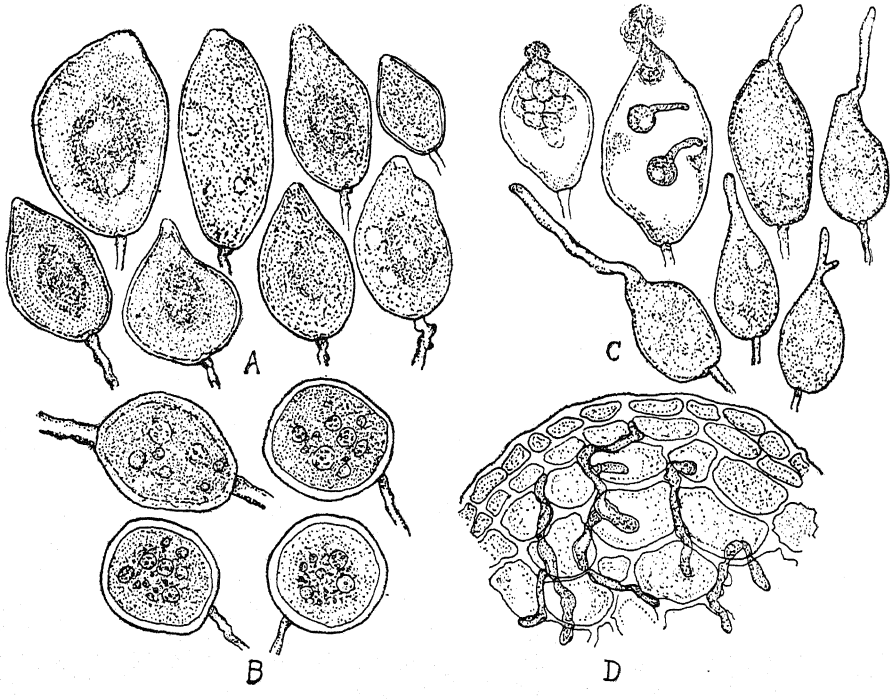


FIG. 2. (*a*) A *mosambi* fruit showing an oily spot due to infection. (*b*) and (*c*) Note mycelial growth on the surface of affected fruits after they have been kept under a bell-jar for a few days.



Phytophthora palmivora.

FIG. 3. *A*. Mature sporangia of common shapes and sizes each showing a prominent papilla of dehiscence. *B*. Intercalary and terminal chlamydospores. *C*. Sporangia showing characteristic germination by formation of zoospores and by hyphae. *D*. Section through a portion of affected rind showing intercellular and intracellular mycelium.

As the disease progresses, the colour of the affected fruit changes to dull brown. Under field conditions, the fungus has never been observed to produce superficial growth on diseased fruits still hanging on the trees, but growth appears after an affected fruit has been kept under a bell-jar for a few days (Plate XLV, fig. 2 (*b*) and 2 (*c*)). The odour of the affected fruit is very characteristic and quite pleasant, but becomes fetid through the invasion of the fruit by secondary decay organisms. The affected fruit remains firm and intact until secondary organisms have reduced it to a mass of putrefying tissue.

In the Bombay Presidency brown rot is very common on *mosambi*, but has also been noticed on *santra* fruits (*Citrus nobilis* Lour. var. *deliciosa* Swingle). It resembles in all respects the California brown rot of lemon, *Citrus limonia* Osbeck, caused by *Phytophthora citrophthora* (Sm. & Sm.) Leonian [Smith, 1907].

Distribution and economic importance

There are no published records of the occurrence of citrus gummosis in India, which is widely distributed in the Bombay-Deccan. It occurs in a serious form in the Deccan and, although it is very destructive in East Khandesh, its absence in the Central Provinces can only be explained on the basis of unfavourable weather conditions, since Mr. J. F. Dastur in a personal communication reports that the varieties of citrus grown in the Central Provinces either for stock or for fruit are the same as those commonly found in Bombay. The disease has not been reported from Gujarat and the Karnatak. Narasimhan [1927], however, has recorded *Phytophthora arecae* (Coleman) Pethybridge on fruits of *Citrus medica* and *Citrus limonia* in Mysore, but he has not reported whether this fungus can also cause gummosis in citrus trees.

The rapid extension of gummosis in the cortex occurs mainly during wet weather, the injury commencing as the monsoon breaks and continuing till the advent of hot weather. It is particularly severe in months of heavy rainfall and during the cold season when late rains are received in heavy quantities. Under such conditions infection spreads rapidly in the orchards, and also the brown rot of fruits becomes very prevalent.

Gummosis attacks citrus trees of all ages, but is often very destructive in young plantations. On the Deccan Canals the disease appears in more or less serious proportions and the damage may be as high as twenty to twenty-five per cent in many orchards. Table I brings out the extent of damage in *mosambi* orchards at a few centres in the Deccan. At Utran in East Khandesh, gummosis has caused very heavy losses in the largest citrus orchard in the Bombay Presidency. The disease is thus a serious menace to the economic growing of citrus in Bombay, and even the death of a few valuable trees in an orchard may mean a serious loss to a grower.

FIG. 1.

TABLE I

Showing the extent of gummosis in mosambi *trees in the Deccan (survey of 1924-25)*

Place	No. of trees examined	No. of trees infected	Per cent infection
Baramati	500	65	12
Poona	336	75	21
Junnar	800	201	25
Poona	1,121	110	9
Rahata	1,200	256	21
Poona	1,800	108	6
Loni	1,000	60	6

PATHOGENICITY

Isolation.—In the early stages of this work the fungus was isolated from the diseased bark just beyond the invaded region, since in this region the fungus is making the most active growth. It was also obtained from naturally infected *mosambi* fruits. More than 160 isolations have been made during the course of this work, the majority of the isolations being from the diseased fruits. In all these cases a species of *Phytophthora* was obtained in pure culture. The fungus can also be easily brought into culture from *mosambi* fruits kept in contact with wet, infected soil collected from the base of heavily diseased trees. The fungus enters the fruit from the infected soil and may easily be isolated by transferring sterilised bits of diseased rind to tubes of oat-meal agar.

The material for isolation was carefully selected from young lesions on the stem and from infected fruits which were still firm and emitting a characteristic pleasant odour, as it had been the experience of one of us [Kamat, 1927] that, when isolations were made from older lesions on the stem and from fruits which had become soft and fetid, secondary organisms like bacteria and *Fusarium* were sometimes present in the culture besides the usual *Phytophthora*. The material from which isolations were made was first disinfected in a one in 1,000 solution of mercuric chloride for three minutes and washed in several changes of sterile, distilled water. It was then placed in tubes of oat-meal agar and incubated at room temperature of 25° C.

Inoculations.—Several young *mosambi* plants in pot-cultures were inoculated with two-week-old cultures of the *Phytophthora* on citrus grown on oat-meal agar. A T-shaped slit was made with a sharp knife in the bark at the bud union, and bits of mycelium from young cultures were applied to the cut surface under aseptic conditions. A similar slit was made on the other side of the union, but no mycelium was applied in this case. Cotton wool was wrapped over these slits and was kept

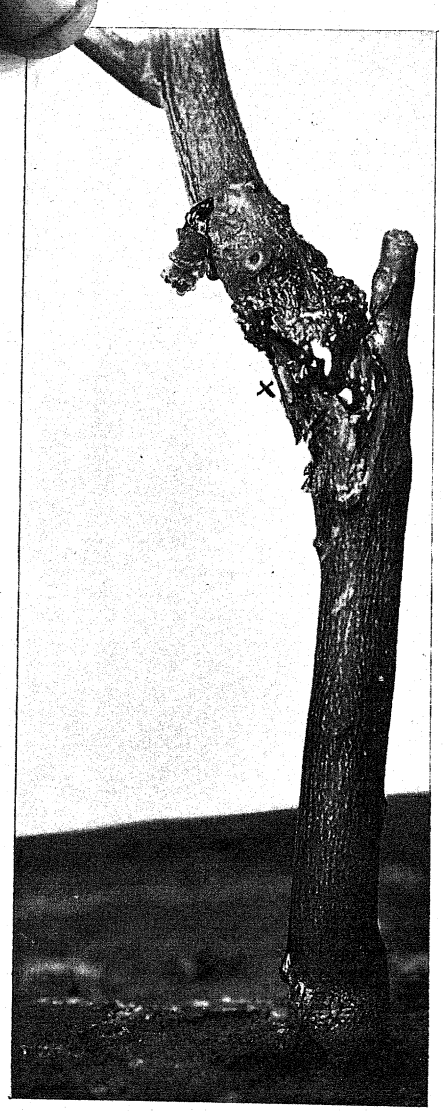


FIG. 1. A two-year old *mosambi* tree inoculated at bud union. Note active oozing out of gum at the inoculated spot marked "×".

FIG. 2. An artificially infected *mosambi* tree showing yellowing and drooping of leaves on the inoculated side.

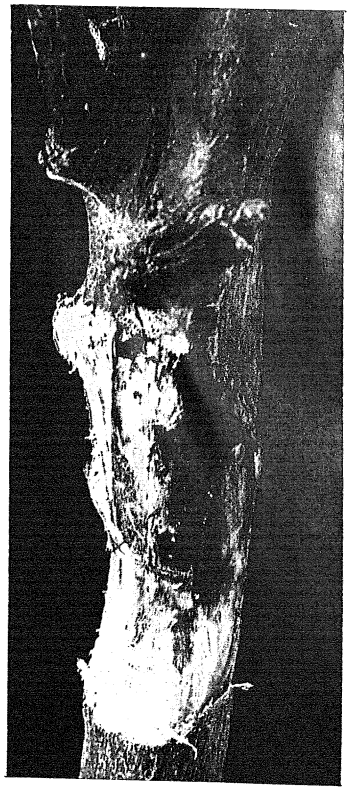


FIG. 3. Same as Fig. 2. showing the area of discoloured bark.

moist with sterile water. At the end of a week cotton was removed, and daily observations were recorded for the period of the experiment. Two of these experiments are described below.

On May 20, 1926, a two-year old *mosambi* tree budded on a *jamburi* stock, a horticultural variety of *Citrus limonia* Osbeck, was inoculated in the above manner at a point a little above the union. On May 31, gum drops appeared on the slit and the bark around the inoculated region had turned brown. After another week these symptoms became very pronounced until, on June 14, gum was actively oozing out of the slit (Plate XLVI, fig. 1), and the area of discoloured bark was $4 \times 1\frac{3}{4}$ cm. (Plate XLVI, fig. 3). The leaves on the inoculated side of the tree were yellow and drooping (Plate XLVI, fig. 2). A similar experiment was started on May 26, and the first signs of infection were visible on June 11. By June 15, gum was exuding copiously and the extent of discoloured bark was $3 \times 1\frac{1}{2}$ cm. The leaves on the inoculated side of the tree were drooping, whilst those on the opposite side were healthy. The fungus was recovered in re-isolation in both cases, and the control cuts remained healthy and healed normally without exhibiting any signs of infection.

Many similar inoculations were made, and the results of some of these tests are recorded in Table II. In all cases including those not mentioned in the table, inoculation with a pure culture of the fungus resulted in positive infection.

TABLE II

Results of inoculation of mosambi *trees with* Phytophthora *sp.*

Point of inoculation	Date of inoculation	Date of observation	Extent of gum lesion	Amount of gum formation
	1926	1926		
Bud union	20th May	24th July	6 × 3	Moderate
Bud union	1st June	20th Aug.	5 × 3	Moderate
Bud union	1st ,,	20th ,,	10 × 3	Abundant
Bud union	26th July	5th Oct.	4 × 2·5	Moderate
Bud union	26th ,,	26th Sept.	3 × 2	Moderate
Bud union	3rd Aug.	5th Oct.	5 × 3	Abundant
Bud union	26th July	5th ,,	6 × 2·5	Abundant
Bud union	3rd Aug.	5th ,,	4 × 3	Moderate
Bud union	6th Aug.	10th Sept.	4 × 3	Abundant
	1928	1928		
Bud union	6th Aug. 1929	10th Sept. 1929	12 × 3	Abundant
Controls	..	..	*Nil*	*Nil*

In another experiment the results of which are shown in Table III, inoculum was applied to slits made on different parts of a *mosambi* plant budded on a *jamburi* stock. The results indicate that the part of *Citrus sinensis* most susceptible to infection is the bud union, although inoculations also succeeded on aerial branches

with slight gumming in a few cases. The roots and stock of *jamburi* were completely resistant, and this explains the common observation in the field that infection rarely travels downwards from the bud union.

TABLE III

Relative resistance of different parts of mosambi *to* Phytophthora *sp.*

Part of plant inoculated	Date of inoculation	Date of observation	Extent of lesion in cm.	Amount of gum formation
Stock, 4in. above ground . .	3rd Aug. 1926 .	5th Oct. 1926 .	2×1·5	*Nil*
Aerial branches .	22nd Aug. 1928	10th Sept. 1928	*Nil*	Very slight
Stock, just below bud union.	3rd Aug. 1929 .	21st Aug. 1929	*Nil*	*Nil*
Roots just below the soil.	5th July 1926 .	25th Sept. 1926	*Nil*	*Nil*
Stock, 6 in. above ground.	27th July 1928	10th Sept. 1928	Very slight	*Nil*
Bud union . .	6th Aug. 1928 .	10th Sept. 1928	12×3	Abundant

RELATIVE RESISTANCE OF CITRUS TO GUMMOSIS

It is a common observation that different species of Citrus show variation in resistance to gummosis under field conditions. Some of the species are completely susceptible, whilst others exhibit resistance of a very high order almost approaching immunity. The information on the relative resistance of species of Citrus is therefore of great practical value, since the use of resistant stock is an important method of preventing gummosis.

The species and varieties of Citrus tested were those commonly grown or used for stock in the Presidency. Young plants of these species were grown in pots and inoculated with bits of mycelium inserted into slits cut in the bark and afterwards covered with wet cotton wool, which was removed after a week. The inoculated plants were kept in a shaded greenhouse and examined after definite periods when the length and width of discoloured bark were measured in each case. The results are summarised in Table IV.

TABLE IV

Relative resistance of different species of Citrus to mosambi Phytophthora

Species of Citrus inoculated	Point of inoculation	Date of inoculation	Date of observation	Extent of lesion in cm.	Amount of gum formation	Remarks
5-year old santra, *Citrus nobilis* var. *deliciosa.*	Bud union .	15th June 1926	20th Aug. 1926	2·5×1·5	Very slight	Strong tendency to be self-limited
5-year old santra, *Citrus nobilis* var. *deliciosa.*	Bud union .	15th June 1926	20th Aug. 1926	2·5×2	Very slight	Strong tendency to be self-limited
2-year old santra, *Citrus nobilis* var. *deliciosa.*	Bud union .	27th July 1928	25th Aug. 1928	Very slight	Very slight	No progress
2-year old pomelo, *Citrus grandis.*	Bud union .	27th July 1928	10th Sept. 1928	4×3	Abundant	Rapid progress
2-year old pomelo, *Citrus grandis.*	Stock .	22nd Aug. 1928	10th Sept. 1928	*Nil*	*Nil*	No infection
2-year old santra, *Citrus nobilis* var. *deliciosa.*	Just below bud union .	3rd Aug. 1929 .	26th Aug. 1929	*Nil*	*Nil*	No infection
2-year old santra, *Citrus nobilis* var. *deliciosa.*	Aerial branches.	22nd Aug. 1928	10th Sept. 1928	*Nil*	*Nil*	No infection
1-year old jamburi, *Citrus limonia.*	6 in. above ground.	27th July 1928	10th Sept. 1928	*Nil*	*Nil*	No progress; wound healed normally.
1-year old kagdi lemon, *Citrus aurantifolia.*	6 in. above ground.	27th July 1928	10th Sept. 1928	*Nil*	*Nil*	No infection
2-year old mosambi, *Citrus sinensis.*	Bud union .	3rd Aug. 1928 .	21st Aug. 1928	5×3	Moderate .	Rapid progress

The results indicate that the species of Citrus grown in Bombay can be grouped into three classes on the basis of their resistance to gummosis. The pomelo and *mosambi* are uniformly very susceptible, and in these species the lesion enlarges rapidly accompanied by profuse gumming. The *mosambi*, *Citrus sinensis* Osbeck, showed such a high degree of susceptibility to gummosis that the area of the lesion was 5×3 cm. in eighteen days after inoculation. Next in the order of resistance is the common mandarin or *santra*, *Citrus nobilis* Lour. var. *deliciosa* Swingle, in which the extent of the lesion produced is limited and there is very slight gum formation ; the inoculated trees show a strong tendency to form a callus tissue along the edges of the dead bark. The *kagdi* lemon, a horticultural variety of *Citrus limonia* Osbeck, may be considered almost immune from gummosis, since the disease makes little progress and there is rapid callus formation and the wound heals completely. These results are thus in accord with the general horticultural practice to graft the sweet susceptible *mosambi* on the sour resistant stock of *jamburi*.

The causal fungus

Morphology.—The mycelium is unseptate, sparingly branched and granular. On corn-meal agar the mycelium makes moderate aerial growth. Within the rind the mycelium is not so irregularly shaped as on culture media, and is mostly inter-cellular, sometimes intra-cellular without haustoria (Plate XLV, fig. 3D). On potato-dextrose agar there is practically no aerial growth, but the mycelium within the medium is stout and irregularly shaped. On oat-meal agar there is also little aerial growth but better than on any of the media tested.

The relation of temperature to growth on oat-meal agar was tested, and cultures were incubated at 4·5, 7·5, 11·5, 17, 20, 26-27, 30, 32·5 and 35° C. in the cold storage rooms of the Fruit Research Station at Ganeshkhind. The tests were run in triplicates, and the results in Table V are the average of the growths in the plates incubated at each of the above temperatures. Growth was measured after eight days.

Table V

Effect of temperature on growth of mosambi Phytophthora *on oat-meal agar*

Temperature (°C.)	Mean diameter of colonies in cm.
4·5	0
7·5	trace
11·5	1
17	6.9
20	8.5
26-27	9.7
30	9.3
32·5	4.5
35	0

The fungus grew abundantly at 27 to 30° C., but failed to develop at 35° C. At lower temperatures, it made very slight growth at 7·5° C., but failed to grow at 4·5° C. The rate of growth at different temperatures is illustrated in Fig. 1.

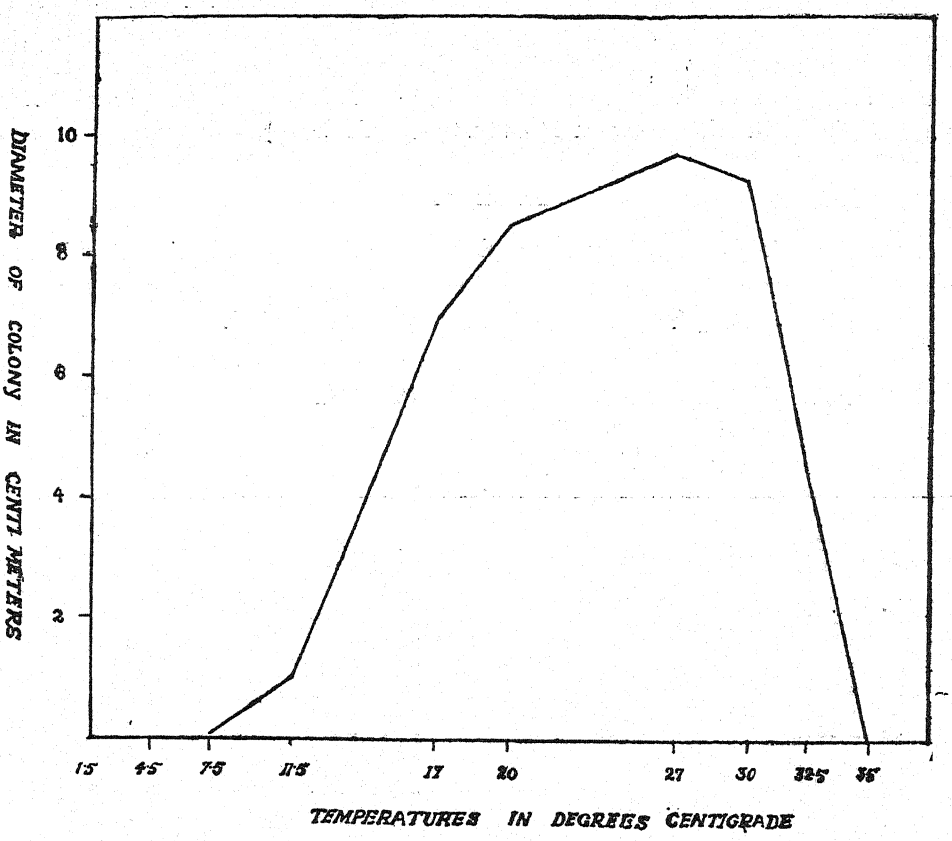


Fig. 1. Showing the effect of temperature on the growth of *mosambi Phytophthora* on oat-meal agar.

Sporangia are completely absent on potato-dextrose agar, but are fairly abundant on one-week-old cultures of oat-meal agar. Corn-meal agar was not as favourable for sporangium formation as oat-meal. The sporangia are large, terminal and lemon-shaped with a prominent apical papilla of dehiscence (Plate XLV, fig. 3 A). They are extremely variable in size and measure from 23 to 79μ in length, with the length mode falling in the class between 53 to 54·9. The range of variation in width, however, is not large, since about seventy per cent of sporangia fall in the class between 25 and 28·9μ (Table VI).

TABLE VI

Measurements of sporangia of Phytophthora *causing gummosis of* mosambi

Length		Width	
Classes in μ	No. of sporangia in 400	Classes in μ	No. of sporangia in 400
21 to 22·9	0	15 to 16·9	0
23 to 24·9	2	17 to 18·9	9
25 to 26·9	3	19 to 20·9	10
27 to 28·9	6	21 to 22·9	22
29 to 30·9	8	23 to 24·9	31
31 to 32·9	8	25 to 26·9	160
33 to 34·9	9	27 to 28·9	119
35 to 36·9	9	29 to 30·9	27
37 to 38·9	10	31 to 32·9	14
39 to 40·9	10	33 to 34·9	8
41 to 42·9	11	35 to 36·9	0
43 to 44.9	14		
45 to 46·9	15		
47 to 48·9	23		
49 to 50·9	41		
51 to 52·9	54		
53 to 54·9	60		
55 to 56·9	29		
57 to 58·9	20		
59 to 60·9	17		
61 to 62·9	16		
63 to 64·9	10		
65 to 66·9	7		
67 to 68·9	7		
69 to 70·9	4		
71 to 72·9	3		
73 to 74·9	2		
75 to 76·9	1		
77 to 78·9	1		
79 to 80·9	0		

Sporangia are produced sparingly in culture, but they are formed freely when young mycelium from a rich medium, such as prune juice, is transferred to sterile blotting paper floating in water in a Petri dish. Under these conditions sporangia are produced in abundance within twenty-four hours. Recently Fawcett and Klotz [1934] have described a somewhat similar method for inducing abundant production of sporangia in *Phytophthora*.

Germination of mature sporangia was studied in water cultures and is much influenced by temperature. The figures in Table VII are the average of three tests.

TABLE VII

Effect of temperature on germination of sporangia of mosambi Phytophthora

Temperature (°C.)	Per cent of germination	
	Direct	Indirect
1·5	0	1·2
4·5	0	6·3
7·5	3·1	15·3
11·5	6·9	33·9
17	12·7	48·6
20	16·1	54·7
26-27	47·6	14·7
30	61·9	4·7
35	20·3	0

The sporangia produce zoospores at 1·5°C., and the amount of indirect germination increases as temperature rises, and is at its optimum at 20°C.; it falls off sharply with further rise in temperature until it is replaced completely by direct germination at 35°C. The sporangia also germinate by the production of germ-tubes, and the optimum temperature for this type of germination is 26-27°C. They do not produce germ-tubes below 7·5°C., but do so readily at 35°C., at which temperature the fungus fails to make any mycelial growth on oat-meal agar (Fig. 2).

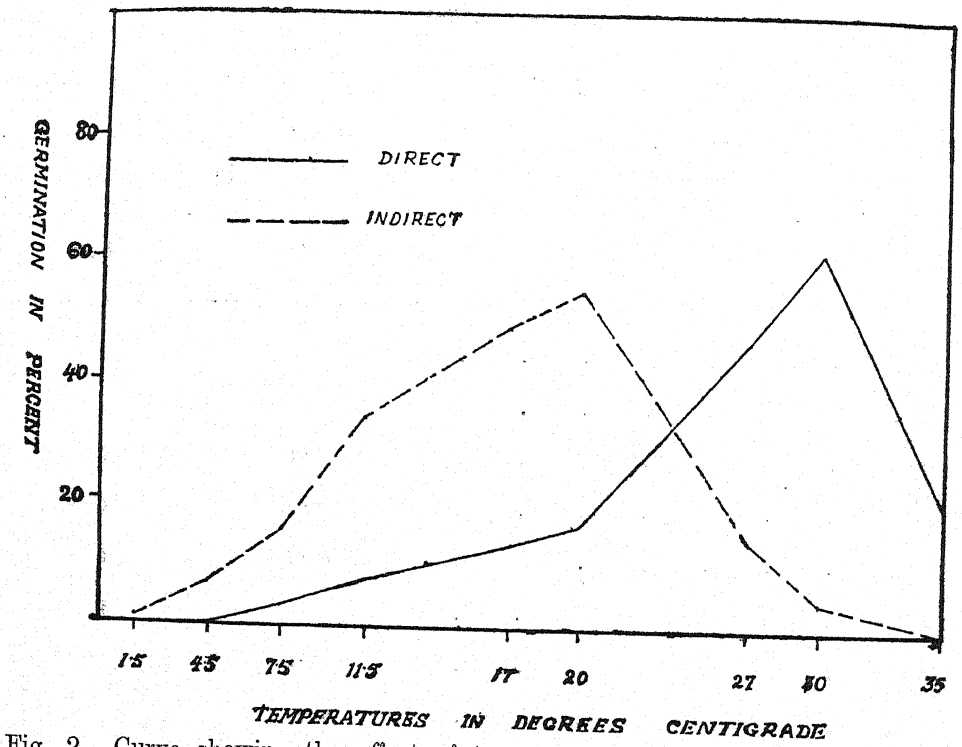


Fig. 2. Curve showing the effect of temperature on the germination of the sporangia of *mosambi* Phytophthora in water cultures.

The fungus does not form oogonia in culture, but produces chlamydospores in large numbers in cultures about two weeks old on oat-meal or corn-meal agar. They are pale yellow, spherical or sub-spherical, mostly terminal but sometimes intercalary, thick-walled and granular, and germinate by one or more germ-tubes (Plate XLV fig. 3 B). In size they range from 12 to 43μ, a majority falling in the class between 29 to 35μ (Table VIII).

TABLE VIII

Measurements of chlamydospores of mosambi Phytophthora

Classes in μ	Diameter: Number of chlamydospores in 400
11 to 12·9	0
13 to 14·9	1
15 to 16·9	11
17 to 18·9	13
19 to 20·9	14
21 to 22·9	16
23 to 24·9	28
25 to 26·9	30
27 to 28·9	30
29 to 30·9	50
31 to 32·9	97
33 to 34·9	61
35 to 36·9	29
37 to 38·9	17
39 to 40·9	2
41 to 42·9	1

TAXONOMY *

In connexion with the identity of this fungus the species of *Phytophthora* that bear close resemblance to the description above given are *Phytophthora citrophthora* (Sm. & Sm.) Leonian, *P. parasitica* Dast. emend. Ashby and *P. Palmivora* Butler. *Phytophthora citrophthora* is very similar to the *mosambi Phytophthora* found in Bombay in respect to the morphology of sporangia and chlamydospores and the type of growth on culture media, but differs from the latter by its lower temperature range, the optimum being between 25° and 27°C. (1931). Moreover, unlike the *mosambi Phytophthora*, *P. citrophthora* does not grow at 32·5°C. but makes slight growth at 5°C. The *mosambi Phytophthora* in Bombay thus differs from *P. citrophthora* in its ability to grow at higher temperatures, a character considered by Tucker [1931] as of specific value and a useful adjunct to morphological characters in the taxonomy of the genus.

* The writers are grateful to Mr. S. F. Ashby, Director, Imperial Mycological Institute, London, for help in the identification of the fungus.

However, the position in respect to temperature relations is reversed when the *mosambi Phytophthora* is compared with *P. parasitica*. The latter develops rapidly at 35°C. (1931), at which temperature the growth of the *mosambi Phytophthora* is completely suppressed. On culture media the *mosambi Phytophthora* makes moderate aerial growth, whilst *P. parasitica* grows very profusely (1931). Besides, the tufted growth of *P. parasitica* serves to distinguish it from the radiate and appressed growth characteristic of *mosambi Phytophthora* (1931).

It will be seen from the above discussion that the *mosambi Phytophthora* can be readily separated from *P. parasitica* on the basis of its inability to grow at 35°C. and the radiate character of its growth. It, however, makes a fairly good growth at 32·5°C., and in this respect resembles *P. palmivora* and differs from *P. citrophthora* ; its optimum temperature for growth also approximates that of *P. palmivora* and is higher than that of *P. citrophthora*. The abundant formation of chlamydospores and in a few cases of sporangia in young cultures and the conidial dimensions are the other two characters which the isolation from *mosambi* has in common with a typical strain of *P. palmivora*. Also like *P. palmivora*, the Bombay fungus does not form oospores in pure culture and, according to Mr. S. F. Ashby to whom a culture was sent for opinion, it falls in the ' rubber ' group of *P. palmivora* as it readily forms these sexual bodies when grown with a member of the ' cacao ' group.

Phytophthora palmivora has not been reported elsewhere* as the cause of gummosis in citrus although Tucker [1931] is of the opinion that this species has probably a wide geographic distribution, and that many of the isolations from citrus in various regions generally referred to *Phytophthora citrophthora* are probably strains of *P. palmivora*. In a letter Mr. Ashby also reports that an identical strain has been isolated by Miss Hellinger from a brown lateral lesion on an orange fruit from Tanganyika Territory in East Africa. It appears, however, that the strain causing gummosis and brown rot of *mosambi* in Bombay is distinct from the one shown by Ocfemia and Rolden [1927] to cause seedling and shoot blight of various species of Citrus in the Philippines.

Control

Prevention.—In the control of citrus gummosis, preventive measures play an important part, and some of the methods in common practice may be briefly discussed.

It has been shown above that some of the sour varieties of citrus, notably *jamburi*, a horticultural variety of *Citrus limonia*, are highly resistant to gummosis, and that the causative fungus enters the tree at the bud union. It is therefore essential to graft pomelo or *mosambi* on a resistant stock at least six inches above

* After the paper was submitted for publication, Briton-Jones and Baker reported *Phytophthora palmivora* as the cause of gummosis in citrus in Trinidad. (*Tropical Agriculture* **12** : 119-125. 1935).

the ground. This method was first introduced in Italy and is now commonly practised in California and other citrus-growing regions.

Soil should not be piled up around the trunk to prevent the collar from too long contact with irrigation water. This practice is very important in orchards where citrus trees are budded low or planted deep in the soil, since the causal organism enters readily at the bud union from the soil harbouring infection. The earth should therefore be removed at the base of the trunk and piled into a small encircling mound so as to prevent both soil and irrigation water from coming into direct contact with the trunk.

Excessive flooding of gardens should be avoided as this tends to create water-logged conditions in heavy soils.

Treatment.—When citrus trees are affected, the only feasible method of treating gummosis is the excision of the diseased bark. Treatment, however, is difficult if the disease is in an advanced stage, although gummosis can be successfully treated in early stages. Before describing experiments on the effectiveness of fungicides tested, the method of treatment may be briefly discussed.

The bark should be scraped until the extent of the diseased patch is determined. The affected bark should then be isolated by cuts extending to the wood, taking care that no injury is caused to the wood by this operation. The cuts should be extended at least half an inch on all sides beyond the limits of the diseased zone. The affected bark should then be removed and the wound covered with twenty-five to thirty per cent creosote oil, which should be allowed to penetrate into the wood and the bark at the sides of the wound so as to kill the fungus. After the surface of the wound is dry, it may be painted with coal tar to protect the exposed wood from the wood-rotting fungi.

If the diseased bark is not carefully removed, the disease may re-appear at the side of the wound, which will require further treatment. Growers are therefore advised to inspect the treated trees periodically to see whether the treatment has been successful.

Fungicides

These experiments were undertaken in the search of a fungicide which, besides being a good disinfectant, is easy to apply and penetrates readily into the wood and the bark at the cut surface of the wound. A brief description of these experiments follows.

Experiments in 1924-26.—The fungicide tested was crude liquid carbolic acid diluted with an equal amount of water, and the results have already been reported separately by one of us [Kamat, 1927], but are briefly summarised here to facilitate reference. About 133 diseased *mosambi* trees were treated in 1924-25, out of which 120 trees were completely cured. The appearance of gummosis in a few cases was probably due to the failure to remove diseased bark completely. The results are shown in Table IX.

TABLE IX

Experiments on the treatment of gummosis with 50 per cent carbolic acid during 1924-26

Place	Number of trees treated	Number of trees cured	Per cent of success
	1924-25		
Baramati	60	55	91·6
Poona	73	65	89·0
	1925-26		
Junnar	201	200	99·5
Rahata	252	245	95·2

Since carbolic acid seemed promising, extensive trials were made in 1925-26 at Junnar and Rahata. The results in Table IX will show that 444 trees completely recovered out of 453 trees treated for gummosis.

Experiments in 1928-29.—Since carbolic acid was not injurious to the bark when diluted with water, its use was safely recommended to citrus growers in the Presidency. In 1928, however, one of the prominent growers in East Khandesh suffered very heavy losses due to the death of many hundred *mosambi* trees caused by the application of undiluted crude liquid carbolic acid. This fungicide was therefore considered entirely unsuitable in the hands of citrus growers, and the search was again made for an effective treatment incapable of causing injury to the treated trees under any conditions.

The fungicides selected for field trials were Zulite, Florium, Coal-tar Creosote and crude liquid carbolic acid. Zulite and Florium were proprietary compounds and were sent for testing by a commercial firm in Bombay. Creosote and Zulite being insoluble in water were applied in full strength. Florium, however, was used in dilute solutions and also in full strength. Carbolic acid was used as a check. All treatments were made in duplicate, but one set of treated trees was painted with coal tar after the treatment and the other was left uncoated. The results of the field trials in six different centres are recorded in Table X.

TABLE X

Summarised results of experiments with different fungicides in the treatment of gummosis during 1928-29

Treatment	Number of trees treated	Number of trees cured	Per cent of success
Zulite	16	6	37·5
Zulit and tar	13	4	30·8
Creosote	17	4	23·5
Creosote and tar	13	7	53·8
Florium	13	4	30·8
Florium and tar	12	6	50·0
50 per cent Florium	12	5	41·6
50 per cent Florium and tar	10	6	60·0
25 per cent Florium	10	7	70·0
25 per cent Florium and tar	8	7	87·5
50 per cent carbolic acid	9	3	33·3
50 per cent carbolic acid and tar	9	3	33·3
25 per cent carbolic acid	8	4	50·0
25 per cent carbolic acid and tar	9	3	33·3

These results did not indicate any special advantage for any of the fungicides tested over the standard carbolic acid treatment. Dilute solutions of Florium were, however, the least satisfactory.

Experiments in 1929-30.—Since the results obtained in the last year were inconclusive, field trials were continued at Rahata with Florium, Zulite and Creosote oil, which latter is a proprietary compound containing twenty-five to thirty per cent high-boiling compounds of coal tar and was supplied by the Eastern Chemical Company, Bombay. The treated trees were about eight to ten years old. The results are recorded in Table XI.

TABLE XI

Experiments on the treatment of gummosis with different fungicides during 1929-30

Treatmen	Number of trees treated	Number of trees cured	Per cent of success
Creosote oil	7	7	100
Zulite and tar	7	3	42·8
50 per cent Florium and tar	7	5	71·4
25 per cent Florium nd tar	11	8	72·7
50 per cent carbolic acid and tar	10	10	100
25 per cent carbolic acid and tar	10	10	100

Zulite and Florium were again relatively ineffective, but all trees treated with Creosote oil recovered completely. It appears that reosote oil has good

penetrating quality and kills the fungus in the bark at the margin of the wound. It has still another advantage in that the wound covered with Creosote oil does not require to be painted with any other substance.

Experiments in 1930-31.—The results with Creosote oil (25 to 30 per cent) were so promising that it was decided to give this fungicide an extended trial with many other fungicides including four new compounds, namely wood-tar Creosote, Anti-ant, and Pesterine and Colas mixed in equal proportions. Wood-tar Creosote is a product of the Mysore Iron Works derived as a bye-product in the distillation of alcohol from wood. Anti-ant was a proprietary compound supplied by Hardcastle, Waud & Co., Bombay, and was mixed with four parts of kerosene oil before applying to the gummous wound. Pesterine and Colas were obtained from the Burmah-Shell Company, Bombay. Through the generous co-operation of Sheth Manakchand Kisondas, about 300 gum-affected trees were placed at our disposal for treatment in his plantation at Utran in East Khandesh. The new fungicides were also tested at Varkhedi. The results are summarised in Table XII.

TABLE XII

Experiments on the treatment of gummosis with different fungicides during 1930-31

Treatment	Utran		Varkhedi	
	No. of trees treated	Per cent of trees cured	No. of trees treated	Per cent of trees cured
Creosote oil	54	98·2	45	89
Creosote oil and tar	32	100	11	91
Wood ar creosote	13	77	43	44
Woodtar creosote and tar	..	..	10	70
Pasterine and Colas (1 : 1)	5	80	58	38
Anti-ant	..	..	126	91·3
Florium	11	82	..	..
Florium and tar	5	80	..	..
50 per cent Florium	5	80	..	..
50 per cent Florium and tar	4	75	..	..
25 per cent Florium	4	50	..	..
25 per cent Florium and tar	4	75	..	..
Zulite	32	90·7	..	..
Zulite and tar	30	93·4	..	..
50 pe cent carbolic acid	47	93·7	..	..
50 per cent carbolic acid and tar.	26	92·4	..	..
25 per cent carbolic acid	18	94·5	..	..
25 per cent carbolic acid and tar.	10	90	..	..

The results of trials at Utran bring out the high fungicidal value of Creosote oil, in which case only one tree failed to recover out of eighty-six trees treated with this fungicide. They also confirm the observation that painting with coal tar of wounds covered with Creosote oil can be dispensed with without serious disadvantage. The results of trials at Varkhedi, however, have little significance since the trees were badly affected before the treatment was applied, and in some cases the disease had almost girdled the trees.

Experiments in 1931-32.—Encouraged by the results obtained during the last year, another series of trials was arranged at Belapur and Rahuri with Creosote oil, woodtar creosote, Anti-ant and Pesterine mixed with Colas. The results in Table XIII are in accord with those reported in the previous experiments and clearly establish the superiority of Creosote oil as a fungicide.

TABLE XIII

Experiments on the treatment of gummosis with different fungicides during 1931-32

Treatment	Belapur		Rahuri	
	No. of trees treated	Per cent of trees cured	No. of trees treated	Per cent of trees cured
Creosote oil	65	100	17	100
Woodtar creosote . . .	..	..	33	2·8
Pesterine and Colas (1 : 1) .	..	..	12	83·4
Anti-ant	28	100	..	..

SUMMARY AND CONCLUSIONS

Gummosis produces typical symptoms in *mosambi*, *Citrus sinensis* Osbeck, and affects the trunk. It is characterised by the copious exudation of a resinous gummy substance and the cracking of the bark for considerable distances upwards from the bud union. The serious aspect of the disease is, however, in the lateral extension of the invaded region, which, if not treated, will kill the tree. The causative fungus also attacks *mosambi* fruits (and *santra* fruits lying on the ground) causing brown rot ; the diseased *mosambi* fruits fall to the ground and are reduced to a mass of putrefying tissue through the invasion of secondary decay organisms.

The disease is widely distributed in the *mosambi* orchards in the Bombay-Deccan, but has not been reported elsewhere in this country. The disease spreads rapidly during damp or wet weather.

Gummosis can be produced by inoculating bits of mycelium rom a young culture of *Phytophthora* isolated from an infected fruit, into slits made in the cortical tissue of the stem.

Experiments on the relative resistance of different species of Citrus indicate that the pomelo and *mosambi*, *Citrus sinensis*, are highly susceptible, whilst the *kagdi* lemon, a horticultural variety of *C. aurantifolia*, and *jamburi*, a horticultural variety of *C. limonia*, are almost immune from gummosis. The common mandarin or *santra*, *C. nobilis* Lour. var. *deliciosa* Swingle, occupies an intermediate position.

The morphology of the causative fungus has been described in detail, and the fungus is further compared with *Phytophthora citrophthora* and *P. parasitica*, the cause of citrus gummosis in other countries. It has been shown that the *Phytophthora* causing *mosambi* gummosis in Bombay is a typical strain of *Phytophthora palmivora* Butler in the 'rubber group' of that species.

Grafting of sweet, commercial varieties of citrus on a sour, resistant stock, such as *jamburi*, will prevent gummosis. Both soil and irrigation water should not be allowed to come into direct contact with the bud union, since the fungus enters the cortical tissue at the bud union from the soil harbouring infection.

The relative effectiveness of a number of fungicides in treating gummosis has been tested. The best method of treatment is to remove the bark and to cover the wound with twenty-five to thirty per cent Creosote oil ; the wound may then be painted with coal tar, if necessary.

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A NOTE ON THE HAIRINESS OF COTTON

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The degree of hairiness of cotton is of considerable importance as it has to be " critically studied in the isolation of special varieties and races of cotton "*. Moreover presence or absence of hairs is thought to be associated with resistance or susceptibility to various insect pests, such as Jassids (*Empoasca devastans*), cotton stem borer, etc. Hairy cottons are also supposed to be resistant to Red Leaf Blight in southern India. In the Punjab it has long been known that hairy cottons are to be preferred for growing in localities where Jassids are a serious pest. The investigation of the degree of hairiness of various varieties of cotton is, therefore, of considerable importance.

Indigenous varieties of cotton grown in the Punjab are very hairy indeed, but the acclimatized American strains differ a great deal in hairiness. It is realized that it is not very safe to introduce smooth-leaved American varieties for general cultivation in the Punjab. The determination of the degree of hairiness of all American strains bred at the Cotton Research Station, is, therefore, a routine operation of great importance. During these investigations, certain observations have been made, which, so far as the writer is aware, have not been previously recorded. These will be briefly described in the present note.

PREVIOUS LITERATURE

Before describing the observations made, the previous literature will be briefly reviewed. References to hairiness of cotton are met with mostly in the genetic studies of this plant. Balls [1912] records that hairiness is of several types and is controlled by a number of factors. Kearney [1923] found that the degree of hairiness of petiole was highly correlated with that of the lower surface of the leaf. He observed that the young leaves were much more hairy than the mature ones and in his genetic studies he recorded the hairiness of half mature leaves. Kulkarni and Khadilkar [1929] also studied the inheritance of hairiness in a cross between *G. hirsutum* and *G. barbadense*. They have given rather extensive data on this point, but have not attempted any factorial analysis of the figures. The method of recording the degree of hairiness has also not been given. Harland [1932] has studied the inheritance of hairiness in several species of cotton. He found intimate connection between the hairiness of various organs of the plant.

*" Wild and cultivated cotton plants of the World," by Sir George Watt. Longmans Green and Company, London, 1907 ; p. 59.

From this review of literature it is found that in genetic studies the method of recording the degree of hairiness has not been mentioned by many workers. This, however, is a very important point as will be shown in discussing the present data and it is surmised that by a proper appreciation of the facts brought out here, the analysis of hybrid population for genetic studies will be greatly facilitated.

Material and method

The degree of hairiness of all important new strains of American cotton has been recorded. During 1933 the leaves of various varieties were only examined, but during 1934 the hypocotyledonary region and the cotyledons were also kept under observation.

In the case of hypocotyledonary region small areas of the bark were peeled off and an area of one cm. $\times 1 \cdot 5$ mm. just from below the cotyledonary node was examined under the microscope. Five such areas from different plants were examined before the shedding of the cotyledons and the hairiness was expressed as an average of these five readings. Thus twelve varieties of American and one of *desi* cottons were examined. All American varieties without any exception were found to be non-hairy, while the *desi* variety (15 Mollisoni) had eighty-seven hairs per 15 sq. mm.

The cotyledons were examined under a binocular and both surfaces were scrutinized in all the varieties mentioned above. It was found that both surfaces of the cotyledons of all varieties, including the *desi* which has harirs on the hypocotyledonary region, were absolutely glabrous. This is rather surprising as the veins and leaves of some of these varieties are exceedingly hairy.

It has been reported that hairs on stem and leaves of cotton are deciduous. It was, therefore, thought necessary to fix the age at which counts should be made. It was decided that all counts should be taken at the age of twenty-two days on which day the leaf is fully mature [Afzal, 1933]. It was also considered necessary to confine our attention to the leaves borne directly on the main stem. Five leaves from five different plants were taken at each time and the number of hairs was counted from an area of one sq. cm. from five different places on each leaf and the hairiness was expressed as an average of all these figures.

In order to study the hairiness of a large number of varieties, counts were made only on the 5th, 15th, 25th and 35th leaf on the main stem. The figures are given in Table I. A consideration of these figures reveals that the leaves produced higher up on the main stem are more hairy than those produced lower below. This point is very important as it might afford an explanation of the confusion of the genetic data with regard to hairiness. It is quite conceivable that the hairiness of different plants in various progeny rows was not counted on leaves of similar ages and similar situation on the main stem. This error alone would cause enough discrepancies in the data as to render these unsuitable for factorial analysis.

TABLE I

Average number of hairs per sq. cm. of leaf area

	5th leaf				15th leaf				25th leaf				35th leaf			
	1933		1934		1933		1934		1933		1934		1933		1934	
	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
(1) 4 F	41	41	50	8	494	72	369	99	918	145	805	385	898	257	737	391
(2) 43 F	38	31	26	10	428	169	575	185	630	173	638	241	1272	309	621	232
(3) 38 F	10	7	Leaves dried up		107	15	46	16	389	76	445	131	..	..	459	102
(4) 45 F	..	..	4	3	..	..	28	12	..	..	349	79	..	..	457	140
(5) L. S. S.	..	..	17	6	..	..	393	77	..	..	632	127	..	..	516	123
(6) 289 F	13	11	..	..	112	27	..	..	152	70	..	..	625	73	..	..
(7) N. T. 4 F	18	11	..	..	212	43	..	..	563	71	..	..	599	112	..	..
(8) 1 F	20	8	..	..	197	49	..	..	527	117	..	..	384	56	..	..
(9) 48 F	6	6	..	..	117	25	..	..	374	88	..	..	691	154	..	..
(10) M. P. 289 F	13	11	..	..	91	53	..	..	554	43	..	..	398	147	..	..
(11) K. T. 23	2	1	..	..	121	25	..	..	449	77	..	..	512	65	..	..
(12) K. T. 25	5	3	..	..	13	4	..	..	139	6	..	..	270	37	..	..
(13) 15 Mollisoni	491	392	306	215	693	465	808	487	1396	1135	1079	943	1390	503	1169	746

These figures are, of course, from a few isolated leaves on the main stem and in order to confirm these, counts were made on all the successive leaves. Five plants of 4 F were examined on 27th-29th September 1934 and the figures are given in Table II. It will again be seen that the upper leaves are much more hairy than the lower ones.

TABLE II

Average number of hairs per sq. cm. of leaf area in successive leaves up the main stem

Leaf No.	15 Mollisoni		43 F		4 F			
	1933		1933		1933		1934	
	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
26							518	61
27							559	84
28							587	86
29							544	87
30			477	174			623	105
31							654	117
32							629	110
33							685	109
34							719	128
35			408	109			744	142
36					773	122	777	152
37							679	138
38					760	127	756	155
39			504	194			823	196
40					882	122	886	229
41			494	137	713	111	986	266
42			765	187	1156	142	981	339
43			Innum erable				1068	399
44	1859	1226						
49	1224	890						
54	2077	1294						
59	2796	1736						
64	innum erable							

The relative hairiness of different varieties is also clear from Table I. The *desi* variety, 15 Mollisoni, is very hairy indeed.

Counts of Jassid population were also taken in a few cases and it appears that some correlation does exist between the hairiness of leaf and Jassid population.

SUMMARY

The hypocotyledonary region of all American strains grown in the Punjab was found to be glabrous, but that of *desi* cotton had eighty-seven hairs per 15 sq. mm. The cotyledons of all varieties were devoid of hairs.

It has been found that the degree of hairiness increases from the bottom of the plant to the top in all varieties. It is, therefore, pointed out that in all genetic studies on hairiness of cotton, it is necessary to fix the location of the leaf and its age for hair-counting in a hybrid population, otherwise genetic analysis would be rendered difficult.

ACKNOWLEDGMENTS

This work was carried out as a part of the Punjab Botanical Research Scheme financed jointly by the Indian Central Cotton Committee and the Punjab Government.

I wish to acknowledge the help of my Assistants Messrs. Sant Singh Verma and B. K. Kar in carrying out these studies.

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A NOTE ON THE STERILE HYBRID BETWEEN *NICOTIANA TABACUM* L. AND *N. PLUMBAGINIFOLIA* VIV.

BY

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(With Plate XLVII and one text-figure)

INTRODUCTION

The genus *Nicotiana* has been used very extensively for genetic investigations. With the object of extending knowledge on the compatibility and relationships of the numerous species, a large number of crosses have been made between them by various workers. One of the most widely used species has been *N. Tabacum* probably because it crosses so readily and also perhaps because of its economic importance. East [1928] in his comprehensive account of the genetics of this genus states that one of the few instances where an attempt to cross *N. Tabacum* with another species has resulted in complete failure is that involving *N. plumbaginifolia*. Christoff [1928] obtained a few seedlings from the cross *N. Tabacum* var. *macrophylla* × *N. plumbaginifolia* but they died after producing 4-5 leaves. The reciprocal cross was entirely unsuccessful. Despite these failures, it seemed desirable to attempt the cross again as a study of the important " leaf-curl " disease of tobacco at Pusa indicated that out of a number of species and varieties, only *N. plumbaginifolia* possessed any resistance or tolerance to the disease. If this species could be crossed with a commercial variety of tobacco, then there was hope that it might be possible to transfer the disease-resistance of the former to the latter. The endeavour to cross *N. plumbaginifolia* with the Pusa type H. 156 proved successful and three vigorous hybrid plants were raised to maturity.

It should be pointed out, however, that *N. plumbaginifolia* is closely allied to *N. longiflora* Cav. and is considered by some authors [East, 1928 ; Hollingshead, 1929] to be merely a variety of the latter. *N. longiflora* has been crossed with *N. Tabacum* by Christoff [1928] who obtained only defective seedlings, and by Malloch and Malloch [1924] who obtained a number of seedlings one of which survived long enough to produce flowers. The latter, however, according to the authors' statement, was a weakly plant quite different from the vigorous *Tabacum-plumbaginifolia* hybrids which form the subject of this note. These are sufficiently interesting, in our opinion, to merit a brief description.

The cross was " difficult " and a very large number of pollinations had to be made. *N. plumbaginifolia* was used as the male parent. Two hundred and thirty-nine capsules containing much shrivelled seeds were finally obtained. The seeds were sown in flats in August 1934, and seven seedlings raised therefrom.

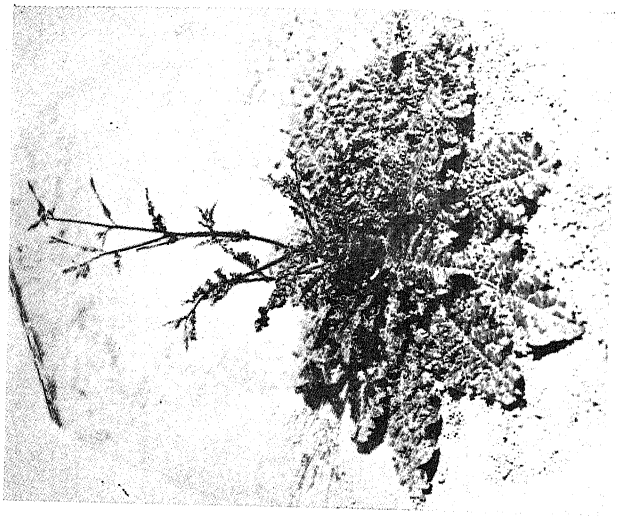

FIG. 3. *N. plumbaginifolia*

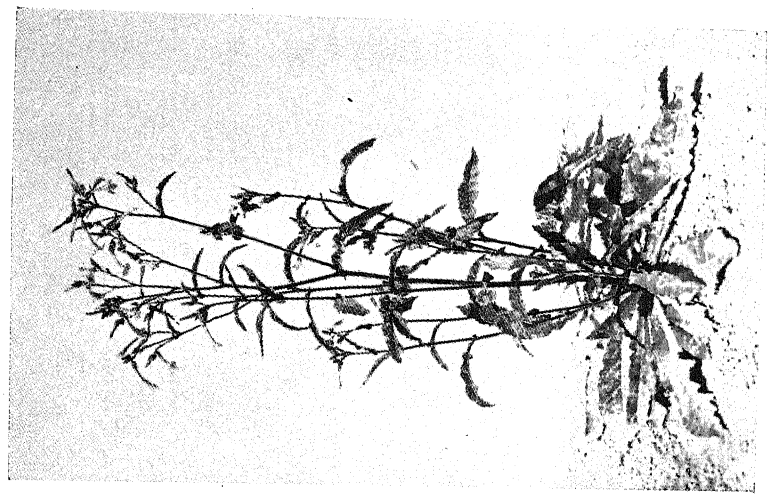

FIG. 2. *N. Tabacum* × *N. plumbaginifolia*—F_1

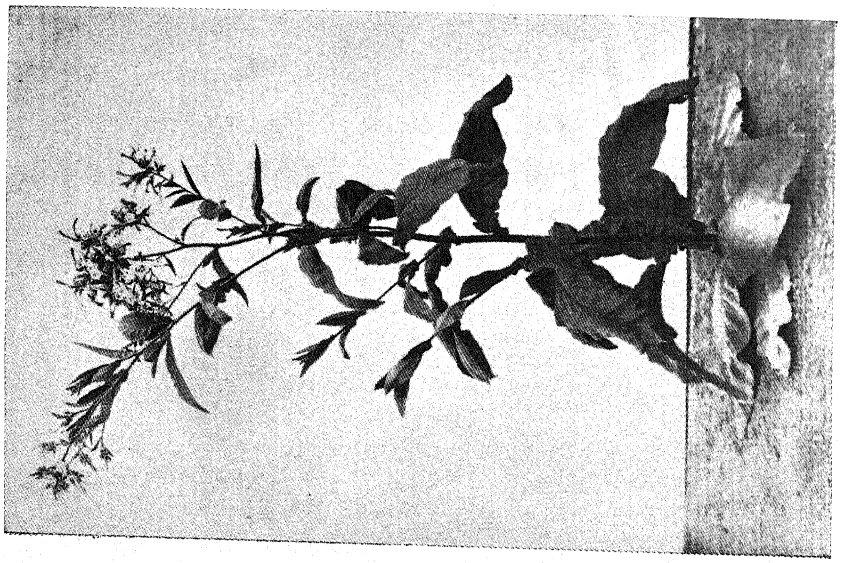

FIG. 1. *N. Tabacum* VAR. PUSA H. 156

The latter were planted out in the field when large enough to handle. They showed marked differences in vigour, and only three reached maturity. These, however, were vigorous, and flowered very freely.

Description of parent varieties and F_1

N. Tabacum *var. Pusa H. 156* (*Plate XLVII, fig. 1*)

Habit.—Plants tall, about 200 cm. high, with long internodes; leaves distributed on the stem at regular intervals.

Leaves.—Sessile, amplexicaul, inserted at an angle of about 75° with the stem; auricles broad and decurrent; lamina ovate; margin entire or slightly wavy; apex acute; surface smooth; texture soft; midrib narrow at base, hairy, smooth; hairs on midrib of one kind with terminal, unicellular, bluntly pear-shaped glands. Foliage colour light green.

Inflorescences.—Terminal open cymes.

Flowers.—Large, remaining open day and night. Pedicel persistent, about 2·0 cm. long, light green in colour. Calyx loose, dilated, more or less globose, light green; 5-fid, lobes triangular, unequal and pointed; ridges not present on calyx tube. Corolla tube broad, abruptly dilating from about the middle; colour white; 5-fid, lobes broadly triangular and faintly marked by a mid-vertical line; orifice about 1·3 cm. in diameter. Inside of corolla base densely hairy. Stamens five, epipetalous; filaments long, fused at base only; anthers large, oval, green.

Ovary conical, green. Style about 4·0 cm. long, thick, usually reaching the level of anthers but remaining a little below the orifice in open flowers. Stigma large, bi-lobed.

N. plumbaginifolia (*Plate XLVII, fig. 3*)

This species is found as a common weed in several parts of India. There appears to be a considerable variation between the different forms. The form used in this investigation is described below.

Habit.—Plants short, about 60 cm. high, with a compact basal rosette of leaves which lie very flat on the ground, throwing up a central floral axis.

Leaves.—Sessile, non-auriculate; lamina lanceolate; margin very wavy; apex acute; surface corrugated; texture hard; midrib broad at base, hairy, rough. Hairs of three kinds present, *viz.*, (*a*) hairs glandular at base, (*b*) hairs with terminal, unicellular globose gland, (*c*) small non-glandular hairs. Foliage colour dark green.

Inflorescence.—A central axis terminated by a very loose cymose inflorescence.

Flowers.—Small, persistent, opening only at night. Pedicel about 0·9 cm. long, purple. Calyx closely enclosing base of corolla tube, dark green with deep purple pigment at base and at tips of lobes; 5-sect, lobes narrow, more or less linear, equal; ten prominent ridges present.

Corolla long, narrow, dilating slightly near the mouth, deep brownish purple with yellow streaks which run down from between the petal lobes on the outside;

5-fid, lobes well-marked, rotate, ovate, marked by a mid-vertical line ; apex acute ; lobe-colour light purple turning to purple in old flowers, colour uniform ; orifice about 0·4 cm. in diameter. Inside of corolla tube base not hairy. Stamens five, inserted directly on throat of corolla tube ; filaments absent ; anthers small, oval-round, deep purple.

Ovary conical, green ; style about 2·7 cm. long, thin. Stigma small, bilobed.

N. Tabacum × N. plumbaginifolia, F_1. (*Plate XLVII, fig. 2*)

Habit.—Plants medium tall, about 80 cm. high, with a loose basal rosette of leaves, and a central floral axis.

Leaves.—Sessile, non-auriculate, slightly raised from ground ; lamina lanceolate ; margin wavy ; apex acute ; surface smooth with slight wrinkling along midrib towards the base ; texture medium hard ; midrib narrow at base, hairy, smooth ; hairs with terminal unicellular oval-round glands. Foliage colour green.

Inflorescence.—A central axis terminated by open cymes.

Flowers.—Medium sized, persisting for three to four days after which they are shed ; they remain open day and night. Pedicel about 1·2 cm. long, green with purple pigment towards base. Calyx loose, dilated, green with purple pigment at base and at tips of lobes ; 5-fid, lobes triangular, unequal, with acute apex ; ridges not present on calyx tube. Corolla tube medium broad, gradually dilating from about the middle ; colour brownish purple on outside, 5-fid, lobes triangular faintly marked with mid-vertical line ; often 1—3 accessory lobes are present ; occasionally more lobes are present. Colour of lobes light purple, often deeper at tips ; with age the colour becomes purple, and small white specks or stripes are often present ; occasionally stripes may be large, involving as much as three-fourths of corolla tube ; very rarely entirely white flowers are produced. Orifice about 0·8 cm. in diameter. Inside of corolla tube base densely hairy. Stamens five, epipetalous ; filaments long, fused for the greater part of the corolla tube ; distance over which fusion occurs is variable, and sometimes the whole of the filament may be fused with the corolla. Anthers medium sized, oval, purple. Pollen hyaline, shrivelled and non-functional. Ovary conical, green. Style about 4·0 cm. long, of medium thickness. Stigma medium-sized, bi-lobed. It will be noted that the hybrid resembles its *Tabacum* parent in respect of width of midrib at base of leaf, hairs on midrib, loose, dilated, 5-fid nerveless calyx, hairy interior of corolla base, oval anthers, and in the flowers remaining open day and night. It resembles the *plumbaginifolia* parent in respect of habit (rosette of leaves with a central floral axis), and in having non-auriculate, lanceolate leaves, coloured base and tips of calyx lobes and purple anthers.

In regard to the following characters, the hybrid is more or less intermediate :– Height of plant, margin surface and texture of leaves, foliage colour, length and colour of pedicel, size of flowers, diameter of orifice of corolla, condition of filaments, size of anthers and stigma.

The following characters are new, not being present in either of the parents :— Streaking and striping of flowers, presence of accessory corolla lobes, complete sterility. The style is longer than in either of the parents.

Discussion

Perhaps the most outstanding characteristic of the hybrid is its complete sterility which probably results from chromosomal unbalance as there is a wide difference in the chromosome number of the parents. *N. Tabacum* has $n = 24$ chromosomes and *N. plumbaginifolia* has $n=10$ [East, 1928]. Back-crossing to each of the parents was tried, and also crossing with *N. rustica* L. ($n=24$), *N glutinosa* L. ($n=12$) and *N. alata* Lk. and Otto ($n=9$), without success. Endeavour was also made to obtain fertility by inducing doubling of chromosome number by exposure to ultra-violet rays* (for $2\frac{1}{2}$, 5, 10 and 15 minutes), exposure to continuous light for ten days, etc. At the end of a year two of the plants died but the third was still growing vigorously and producing numerous flowers at the time of writing this note. Several branches have been successfully grafted on to *Tabacum* stocks and in this way it is hoped to continue the hybrid. Anther material of both parents and the hybrid has been preserved and it is hoped that a cytological investigation will be possible in the future.

An interesting feature is the streaking and striping of the flowers, (Fig. 1), a character not present in either of the parents. Such streaking in species hybrids in *Nicotiana* has been noted by Kostoff [1935] in distant crosses involving the species *Tabacum* and *Sanderae*. He mentions that the streaking increased with the age of the plant, an observation which is in agreement with our experience.

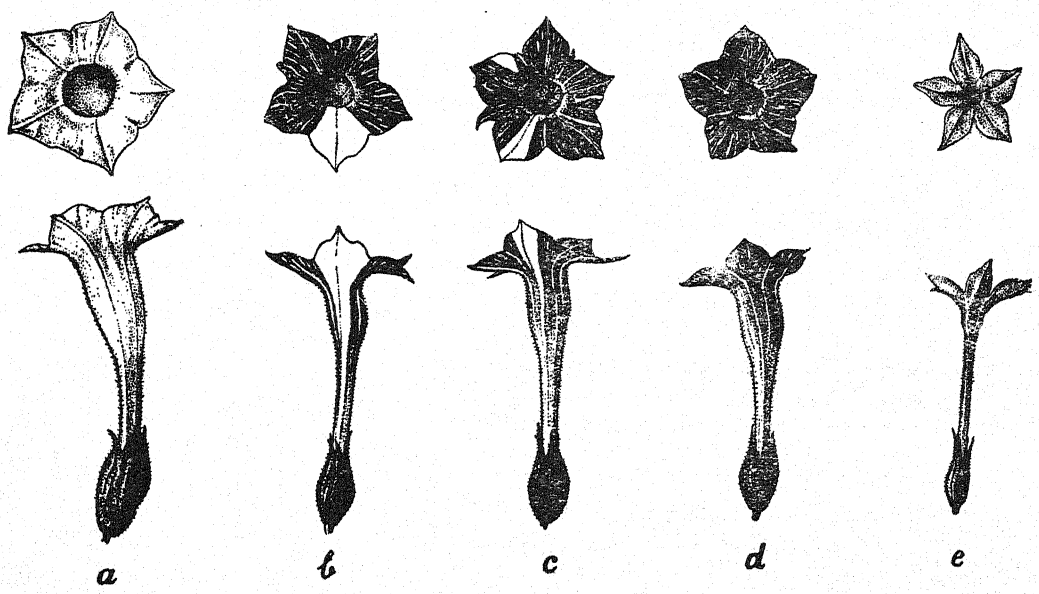

Fig. 1.

Fig. 1. Flowers of *N. Tabacum* (*a*), *N. plumbaginifolia* (*e*), and *N. Tabacum* × *N. plumbaginifolia* F_1 (*b*, *c*, *d*). ($\frac{3}{4}$ nat. size).

* This treatment was kindly carried out by Dr. B. B. Mundkur in the Mycological Section.

The greatest interest of the present cross probably lies, however, in its bearing on Goodspeed and Clausen's reaction-system hypothesis. It has already been noted by a number of investigators [East, 1928 ; Christoff, 1928, etc.] that when hybrids are obtained by crossing species differing widely in their chromosome numbers, they generally resemble the parent having the larger chromosome number. The degree of dominance of the characters of the parent with the larger chromosome number however apparently varies [East, 1928 ; Christoff, 1928 ; Malloch and Malloch, 1924 ; McCray, 1932 ; etc.]. Thus the hybrid between *N. longiflora* and *N. Tabacum* studied by Malloch and Malloch [1924] resembled more the *Tabacum* parent but showed a few *longiflora* characters. The present case is interesting in showing a condition in which the hybrid is intermediate between its parents. The *Tabacum* characters are apparently unable to " dominate " as in other crosses between *N. Tabacum* and species with low chromosome numbers.

Inheritance of nicotine content

Determinations of nicotine content were kindly carried out by the Imperial Agricultural Chemist to whom our thanks are due. The results were as follows.

	Nicotine content (percentage)
N. Tabacum var. Pusa H. 156	1·07
N. plumbaginifolia	0·09
N. Tabacum × *N. plumbaginifolia* F_1	0·80

The hybrid is more or less intermediate, though nearer the parent with the higher nicotine content. This result may be compared with that obtained by Hackbarth and Sengbusch [1935] who found that high nicotine behaved as a simple dominant character to absence of nicotine, the F_1 showing partial dominance of the former. Kostoff and Popoff [1931] however found that in species crosses the nicotine content of the F_1's was lower than the average of the two parents, while the F_2 results indicated the presence of multiple factors.

Summary

The vigorous but sterile hybrid obtained by crossing *N. Tabacum* var. Pusa H. 156 with *N. plumbaginifolia* is described.

The percentage nicotine content of the hybrid was 0·80 as compared to 1·07 of the *Tabacum* parent and 0·09 of the *plumbaginifolia* parent.

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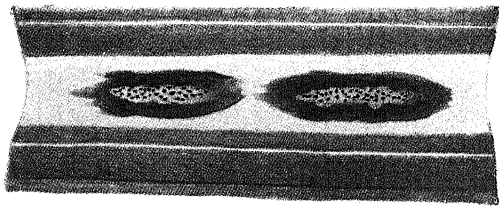
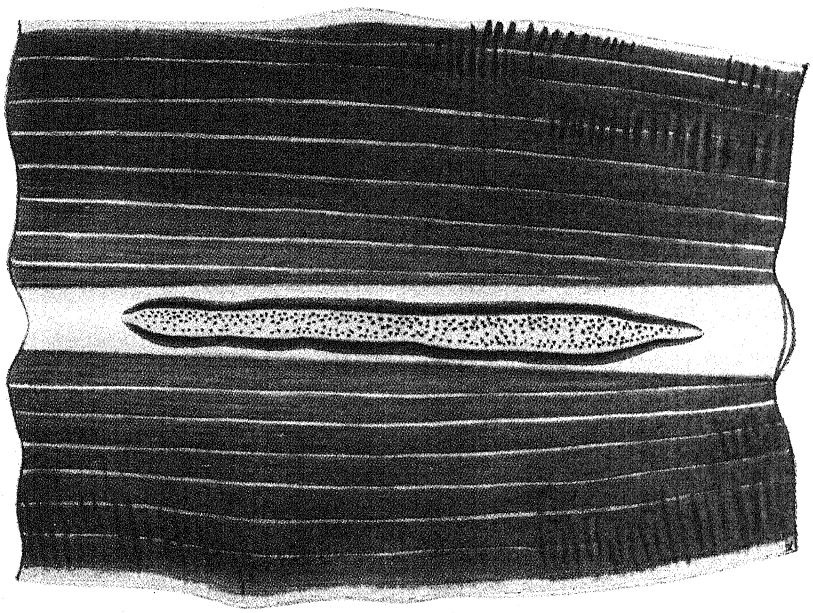
PLATE XLVIII.





Symptoms of the disease (natural size)

A DISEASE OF *ZEA MAYS* CAUSED BY *COLLETOTRICHUM GRAMINICOLUM* (CES.) WILS.

BY

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(Received for publication on 19th February 1936)

(With Plates XLVIII and XLIX and one text-figure)

CONTENTS

		Page
I.	Introduction	833
II.	Morphology of the parasite on the host	834
III.	Parasitism—	
	(*i*) Inoculation on the host	834
	(*ii*) Mode of penetration	835
	(*iii*) Cross inoculations	835
IV.	Growth in culture	835
V.	Factors affecting sporulation	837
VI.	Spore germination	837
VII.	Comparison of *Colletotrichum* sp. on *Zea Mays* with *C. graminicolum* (Ces.) Wils. on *Sorghum vulgare*	841
	(*i*) Inoculation experiments	841
	(*ii*) Morphological characters	841
	(*iii*) Cultural characters	842
VIII.	Conclusion and summary	842
	References	843

I. Introduction

A disease of *Zea Mays* caused by *Colletotrichum* sp. was noticed at Pusa during 1934. The disease makes its appearance first on the central midrib of the leaf in the form of small brownish spots on the upper side. These spots gradually elongate along the long axis of the midrib in both directions, often reaching from the leaf-sheath to the tip of the leaf. The central portions of the spots later on become straw coloured and bear the minute black fruiting pustules of the parasite—the acervuli. Plate XLVIII shows the symptoms of the disease.

Colletotrichum cereale Manns., *C. lineola* Cda. and *C. graminicolum* (Ces.) Wils., all of which are now considered synonyms, have been reported parasitic on *Zea Mays* from North America [Seymour, 1929]. Recently a new disease of *Zea Mays* has also been reported from Russia and ascribed to *C. zeae* nov. sp. [Lobik, 1933].

II. Morphology of the parasite on the host

Mycelium.—The mycelium of the parasite consists of slender, branched, colourless, septate haphae. These are both inter and intra-cellular and ramify in the parenchymatous tissue ; sometimes they are also observed in the vascular bundles.

Acervuli.—The acervuli (Plate XLIX, fig. 1) develop late in the season. They appear below the cuticle in stromatic masses and soon rupture the cuticle. When mature they are circular or oval, varying greatly in diameter.

Setae.—Setae appear in mature acervuli. They are mixed with the conidiophores in the acervulus and are long, dark-brown to black, thick-walled processes or bristles, somewhat pale at the tip. They are unbranched, 2 to 5-septate and measure from 4-6μ in width and up to 170μ in length. Usually the setae are broader at the base, often bulbous and gradually tapering towards the apex.

Conidiophores.—Some of the superficial cells of the stromatic mass which develops by the aggregation of fungal hyphae grow out and form the setae while the others remain short and colourless and form a crowded layer—the conidiophores—bearing the conidia at the tip. They are broader at the middle than at the ends and measure 2-7 $\times$ 1-2μ.

Conidia.—The conidia are hyaline, falcate, with a single or sometimes two oil globules and measure 18-31 $\times$ 4-5μ (Plate XLIX, fig. 1).

III. Parasitism

(*i*) *Inoculation on the host.*—Single spore cultures of *Colletotrichum* sp. were obtained by the usual plating method and a series of inoculation experiments were carried out on maize plants both in the laboratory and in the field by placing spores and mycelium on unwounded host parts or by spraying with spore suspensions in sterile water. The inoculated plants were kept in moist chambers for twenty-four hours, later they were kept in the open but always kept moist by occasional spraying with water. The results of the inoculation experiments are summarised in Table I. It will appear from the table that the fungus is a parasite, capable of attacking all parts of the host plant.

Table I

Summary of the inoculation experiments carried out with Colletotrichum *sp. on* Zea Mays

Parts of the plant inoculated	No. of plants inoculated	No. of plants infected	No. of controls kept	No. of controls infected	Remarks
Leaves(both sides)	97	94	20	Nil	Plants kept 24 hours in moist chambers after inoculation, later in the open.
Midrib . .	89	87	32	,,	
Leaf-sheaths. .	75	70	25	,,	

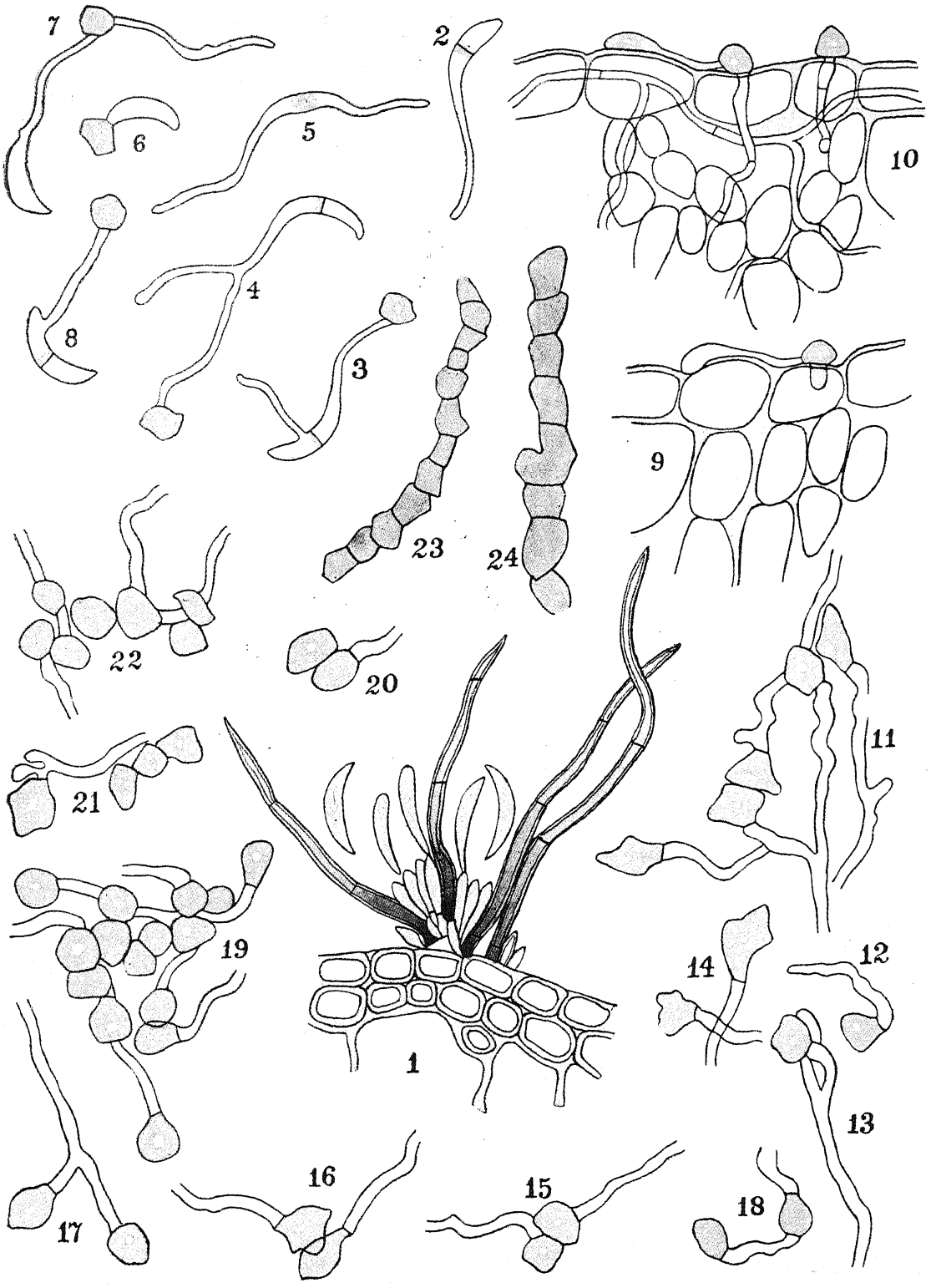

FIG. 1. Acervulus showing conidiophores, conidia and setae (×420).

FIGS. 2-8. Germination of conidia and formation of appressoria (×420).

FIGS 9 & 10. Germination of conidia on the epidermis of the leaf and the mode of penetration (×420).

FIGS. 11-17. Appressoria formed in seven days old culture (×420).

FIGS. 18-22. Appressoria in 14 days old culture (×420).

FIGS. 23 & 24. Appressoria in chains in old culture (×420).

(*ii*) *Mode of penetration.*—Within twenty-four hours after inoculation a great majority of the fungus spores germinate (Plate XLIX, figs. 2-8) giving out one or two germ-tubes. These germ-tubes on coming in contact with the epidermis of the host form appressoria. These appressoria in turn germinate in the same manner as the conidia (Plate XLIX, figs. 9 and 10). The appressorium becomes thinner and colourless on one side and from this spot a germ-tube appears. The germ-tube penetrates directly through the cuticle into the epidermal cell, probably by mechanical pressure as reported by Dey [1919] for *C. lindemuthianum* (Sacc. & Mag.) Br. & Cav. Stomatal entrance or penetration through wounds has not been noticed, though it is not impossible. In no case has penetration been observed except from an appressorium.

(*iii*) *Cross-inoculations.*—Numerous cross-inoculations with the fungus on a number of other related plants were made with a view to determining the possible host range by placing spores and mycelium on unwounded parts. The details of the cross-inoculation experiments are given in a tabulated form in Table II. It will appear from the table that the fungus is capable of infecting a wide range of hosts under laboratory conditions.

TABLE II

Summary of cross-inoculation experiments on various hosts with Colletotrichum *sp. from* Zea Mays

Host	Number of inoculations	Number of plants infected	Percentage of infection
Panicum typhoideum . .	35	32	91·4
Sorghum vulgare . . .	50	48	96·0
Eleusine indica . . .	48	42	87·5
Setaria italica . . .	30	25	83·3
Euchlœna mexicana . .	46	40	86·9
Panicum frumentaceum . .	49	48	97·9
Paspalum scrobiculatum . .	28	24	85·7
Hordeum vulgare . . .	37	35	94·5
Avena sativa	58	56	96·5
Triticum vulgare . . .	46	45	97·8

IV. GROWTH IN CULTURE

The fungus was grown on a large number of media. The best aerial growth with copious sporulation took place on maize-meal, oat-meal, Dox's and Hopkin's agars. Brown's synthetic agar gave a poor aerial growth and sporulation. Scanty aerial mycelium and poor sporulation were obtained on maize-leaf juice agar,

maize-stem juice agar, *juar*-leaf juice agar and potato agar. Growth and sporulation were the poorest on nutrient agar. Sterilised maize stems formed a very suitable medium for copious spore formation.

In young cultures the mycelial threads are hyaline, small, septate and much branched. But gradually they darken, grow broader and become thick-walled.

In cultures three or four days old, conidia are produced at the ends of hyphae. The conidium is at first continuous with the hypha from which it is developed ; it grows in size and is then cut off from the hypha by the formation of a septum at the constricted part. The hypha then elongates and a second spore is formed. In this way several conidia are produced from a single hypha which in mass appear pinkish. Similar conidia formation in culture also occurs in other species of *Colletotrichum* [Edgerton, 1910 ; Barrus, 1921].

Acervuli are not always formed in culture. On maize and oat-meal agars they have been obtained though not in large numbers. They are similar to those formed on the host in nature. The setae are dark brown to black, light coloured at the tip, unbranched, 2-5 septate, broader (sometimes bulbous) at the base and gradually tapering towards the apex, measuring up to 170μ in length and 4-6μ in width. The conidiophores are crowded, hyaline and measure 2-7 $\times$ 1-2μ. The conidia are falcate, hyaline with one or two oil globules measuring 17-32 $\times$ 4-6μ in diameter.

Appresoria are formed in great abundance in cultures (Plate XLIX, figs. 11-22). Their number is more when the fungus sporulates poorly and does not form acervuli in culture. The appressoria are dark brown in colour and the great majority of them are simple spherical or pyriform in shape and about 8-15μ in diameter. When they are of irregular shape they may be larger. They are usually seen to develop in contact with the hard surface of the glass-tubes. In crowded and old cultures, however, they often form in the substance of the medium entirely, separated from the glass or any solid substance in the medium. Very often the appressorium has a clear white spot near the centre, the so-called "germ-pore" [Edgerton, 1910], but in some it has been found wanting. At times a large number of appressoria are seen to develop in rows (Plate XLIX, figs. 23 and 24).

V. Factors affecting sporulation

Light.—Continuous light or darkness has been found to inhibit sporulation. Cultures kept in alternate light and darkness (day and night) have been found to sporulate earlier and more copiously than those kept in continuous light or darkness.

Humidity.—Conditions of high humidity have been found to favour sporulation. When Petri dishes were grown upside down over a water surface (100 per cent relative humidity) there was more sporulation than in Petri dishes similarly held over different concentrations of sulphuric acid giving relative humidities of eighty and sixty per cent.

Sometimes sporulation has been found to be copious in cultures saturated with water. When bits of agar containing mycelium of the fungus were placed in watch glasses and saturated with distilled water copious sporulation and numerous acervuli formation took place.

Media.—Other factors being the same sporulation varied on different media. In general sporulation was more liberal when the fungus was grown on meal agars than on synthetic media. The most copious spore production, however, took place when the fungus was grown on sterilised maize stems in tubes.

TABLE III

Sporulation of Colletotrichum *sp. on different media*

Sterilised maize stems	Very good
Maize-meal agar	Good
Oat-meal agar	Good
Dox agar	Moderate
Hopkin's agar	Moderate
Brown's synthetic agar	Poor
Potato agar	Poor

Wounding.—Sporulation is accelerated in a culture by local wounding. Petri dish cultures which were wounded here and there were found to sporulate fairly strongly along the lines of wounding.

VI. SPORE GERMINATION

The time required for the germination of spores varies with the culture media used, the age of the spores, temperature, hydrogen-ion concentration, humidity, presence or absence of plant tissue.

Under the most favourable conditions spores may germinate within ten to twelve hours but they usually require somewhat longer, and some do not germinate for days, although to all appearances conditions are favourable.

Germination usually takes place by the protrusion of one or more germ-tubes (seldom more than two) from any portion of the spore (Plate XLIX, figs. 2-8). They arise most commonly from the side and near the end. The size and length of the germ-tubes differ on different media and under different environmental conditions. In nutrient media the germ-tubes commonly grow directly into profusely branching mycelium or form appressoria which in turn germinate and form a second appressorium or grows into a mycelial weft. In non-nutrient solutions appressoria are formed at the ends of the germ-tubes if conditions are favourable for their formation, otherwise the germ-tubes disintegrate without further development. A great many of the spores have been observed to become two-celled before germination.

Sugars.—Sugar solutions have been found to exert a favourable influence on the germination of spores. Spores which germinated very slowly in water, gave a higher percentage of germination when placed in sugar solutions; their germ-tubes also attained greater length.

TABLE IV

Effect of sugar solutions on the germination of spores of Colletotrichum *sp. on* Zea Mays *(based on the measurement of 100 spores)*

Media	8 hours		12 hours		24 hours		48 hours	
	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes
Distilled water .	...	...	...	...	5	15	18	40
Tap water . .	2	12	5	15	19	31	42	75
Five per cent glucose solution.	8	24	25	36	78	97	96	125
Five per cent saccharose solution.	8	24	24	37	77	92	94	120

Humidity.—The effect of different relative humidities on the percentage of spore germination and the length of the germ-tubes were determined by the method suggested by Galloway [1935]. Half inch squares of thin sheet viscose (" cellophane ") which were soaked in one per cent glucose solution, and blotting off the excess of the solution, dried in air were used. Spores of the fungus were placed on these " cellophane " pieces in the form of thin streaks by folding them in half and lightly touching with the folded edge, the sporing surface of sterilised maize stems. These squares were then suspended by loops of threads in stoppered bottles of about 500 c.c. capacity, each containing 30 c.c. of sodium chloride solutions of different concentrations. These bottles were then kept in an incubator at 25°C. For each relative humidity test a large number of " cellophane " pieces were used. No " cellophane " piece was replaced in the bottle after examination. The results obtained are recorded in Table V.

TABLE V

Germination of the conidia of Colletotrichum *sp.* on Zea Mays *at different relative humidities (Data based on the measurement of 100 conidia)*

Relative humidity	16 hours		40 hours		72 hours	
	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ
100·0 per cent . .	56	74	96	82	...	...
96·9 ,, ,, . .	16	25	25	44	45	75
95·0 ,, ,, . .	...	...	4	20	12	48
93·9-87·5 ,, ,, . .	...	...	...	...	...	...

After seventy-two hours it was not at all possible to determine the percentage of germination and the length of the germ-tubes at 100 per cent relative humidity on account of the growth and overlapping of the germ-tubes. It will be evident,

however, from results recorded in Table V that the best germination of the conidia takes place at 100 per cent relative humidity. At lower humidities there is a gradual fall in the rate of germination and no germination takes place below ninety-five per cent relative humidity.

Temperature.—To determine the effect of temperature on the germination of conidia, loops of spore suspension in sterile water were placed on clean cover slips which were then inverted over glass rings cemented to slides. A small quantity of sterile water was placed at the bottom of each cell. These hanging drops—four of each—were then placed in incubators running at different temperatures. The percentage of germination and the average length of germ-tubes were calculated from a count of 100 conidia at random in each cell. The results obtained are recorded in Table VI.

TABLE VI

Effect of temperature on the germination of conidia of Colletotrichum *sp. on* Zea Mays

Temperatures	16 hours		48 hours		Remarks
	Percentage germination	Average length of germ-tubes μ	Percentage germination	Average length of germ-tubes μ	
20°C. . . .	24	18	38	35	Germ-tubes normal
25°C. . . .	36	32	65	56	,, ,, ,,
30°C. . . .	52	46	88	75	Germ-tubes abnormal.
32·5°C. . .	63	52	92	79	,, ,, ,,
35°C. . . .	..	..	..	..	

It will be noticed from the table that the optimum temperature for the germination of conidia lies between 25°C. and 30°C.

Plant tissue.—Several investigators have found that the presence of host tissue accelerates the germination of spores of various pathogens. Anderson [1914] showed it for pycnospores of *Endothia parasitica* (Mur.) Anders.; Whitehead [1920] for spores of *Urocystis cepulae* Frost.; Brown [1922] for conidia of *Botrytis cinerea* Pers.; Leach [1923] for spores of *Colletotrichum lindemuthianum* (Sacc. and Mag.) Br. and Cav.; Noble [1924] for spores of *Urocystis tritici* Koern; Christensen [1926] for spores of *Helminthosporium sativum* P. K. and B.; and Mitra and Mehta [1934] for spores of *H. nodulosum* B. et C. Besides, the experiments of Christensen [1922], Leach [1923], Noble [1924] and Mitra and Mehta [1934] show that tissues of plants other than the host also stimulate the germination of spores.

The effect of plant tissues on the germination of conidia of the fungus were studied by placing conidia in watch glasses containing from 0·5 c.c. to one c.c. of

distilled water. To these watch glasses from ten to twenty small pieces of plant tissues were added. These plant tissues were first carefully washed with 1 : 1000 solution of mercuric chloride and then the last traces of mercuric chloride removed by several washings with sterile distilled water. The controls contained distilled water only. All tests were made in duplicate and at room temperature (about 30°C.) The percentage of germination and the average length of germ-tubes were calculated from a count of 100 spores at random in each dish. The results obtained are recorded in Table VII.

TABLE VII

Effect of plant tissue on the germination of conidia of Colletotrichum *sp. on* Zea Mays

Plant tissue	20 hours	
	Percentage germination	Average length of germ-tubes(μ)
Control	12	60
Zea Mays (boiled)	12	60
Saccharum officinarum	21	87
Brassica campestris	28	109
Glycine hispida	35	129
Eleusine indica	42	131
Triticum vulgare	68	168
Sorghum vulgare	70	168
Panicum frumentaceum	70	167
Zea Mays	72	172

It will be observed from Table VII that the best germination took place in the watch glasses containing the host tissues. Presence of un-related plant tissues also enhanced the germination of spores. In the presence of *Sorghum vulgare, Triticum vulgare* and *Panicum frumentaceum* tissues the condia germinated almost as well as in the presence of *Zea Mays* tissue.

In the control it was noticed that there was a higher percentage of germination of conidia floating on the surface than those submerged in water. On the contrary little or no difference could be observed in the percentage of germination of floating or submerged conidia, in watch glasses containing plant tissues. Christensen [1926] and Mitra and Mehta [1934] have also made similar observations for conidia of *Helminthosporium sativum* P. K. and B. and *H. nodulosum* B. et C. respectively. Brown [1922] found that certain plants produce volatile substances which either stimulate or inhibit spore germination. Christensen [1922] working on *Helminthosporium sativum* concludes " apparently the stimulatory agent is not specific." It was found, however, that boiled leaves of maize were incapable of stimulating

germination; this shows that the stimulatory principle is destroyed by heat. Germ-tubes were found always directed towards plant tissues showing that the plant tissues exerted some " chemotrophic influence " [Mitra and Mehta, 1934].

VII. COMPARISON OF *COLLETOTRICHUM* SP. ON *ZEA MAYS* WITH *C. GRAMINICOLUM* (CES.) WILLS. ON *SORGHUM VULGARE*

(*i*) *Inoculation experiments.*—With a view to determine whether *Colletotrichum* from *Zea Mays* can infect *Sorghum vulgare* and *vice versa*, a large number of cross-inoculation experiments were carried out. The results of the inoculation experiments are tabulated in Table VIII.

TABLE VIII

Summary of inoculation experiments carried out with Colletotrichum *sp. from* Zea Mays *and* C. graminicolum *on* Sorghum vulgare *and* Zea Mays *respectively*

Fungus	Plants inoculated	Number of inoculations	Number of infection	Percentage of infection	Remarks
Colletotrichum sp. from *Zea Mays*	*Sorghum vulgare*	36	36	100	Controls were kept and were intact.
C. graminicolum.	*Zea Mays*	32	32	100	

It will be noted from the above table that the fungus isolated from *Zea Mays* is capable of infecting *Sorghum vulgare* and that *C. graminicolum* isolated from *Sorghum vulgare* is capable of infecting *Zea Mays*. In both the cases all the plants inoculated took infection while the controls remained intact.

(*ii*) *Morphological characters.*—In shape and size the conidia of *Colletotrichum* sp. occurring naturally on *Zea Mays* agree very closely with those of *C. graminicolum* on *Sorghum vulgare*. A measurement of two hundred conidia, each from *Zea Mays* and *Sorghum vulgare* showed that the range of conidia measurements was 18-31 × 4-5μ for *Colletotrichum* sp. on *Zea Mays* and 19-30 × 4-5μ for *C. graminicolum* on *Sorghum vulgare*. The means for lengths and breadths with their standard errors were $24{\cdot}90 \pm 0{\cdot}2261 \times 4{\cdot}502 \pm 0{\cdot}0352$ for *Colletotrichum* sp. on *Zea Mays* and $24{\cdot}70 \pm 0{\cdot}2050 \times 4{\cdot}55 \pm 0{\cdot}0351$ for *C. graminicolum* on *Sorghum vulgare*. It is clear, therefore, that there exists no significant difference between the two fungi from the morphological point of view.

(*iii*) *Cultural characters.*—*Colletotrichum* sp. from *Zea Mays* and *C. graminicolum* from *Sorghum vulgare* were grown on maize-meal agar, oat-meal agar, Dox's agar, Brown's synthetic medium, Hopkin's medium and Coon's agar under identical conditions for comparison.

Colletotrichum sp. from *Zea Mays* resembles *C. graminicolum* in the following :—

(*a*) Production of white cottony or fluffy type of mycelial growth and in the formation of acervuli and appressoria in culture.

(*b*) Similar growth rate at different temperatures and on a variety of media. Fig. 1 which gives the growth rate after seven days on Hopkin's, Brown's and Dox's media show how closely they resemble.

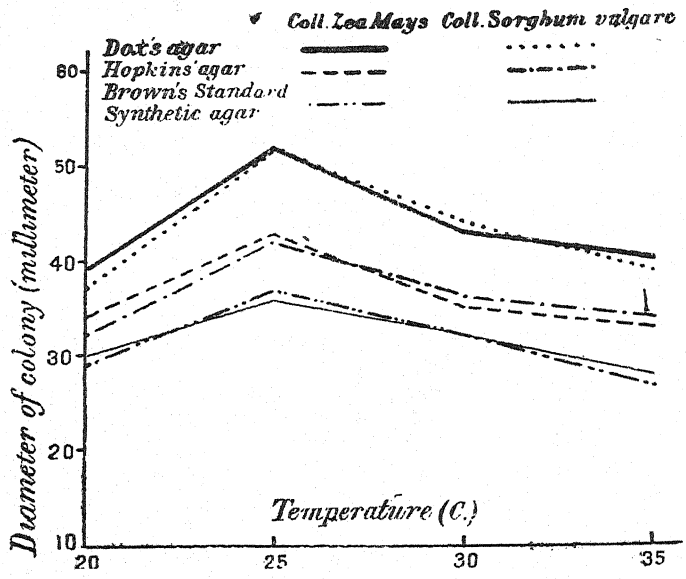

Fig 1. Comparative growth rate of *Colletotrichum* sp. from *Zea Mays* and *Sorghum vulgare* on various media.

Colletotrichum sp. on *Zea Mays* agrees closely with *C. graminicolum* as regards the dimension of spores on different media. Under the same cultural conditions the range of spore length was very close. Two hundred spores were measured at random from oat-meal cultures fifteen days old and grown under the same environmental conditions. The range of spore length and width for *Colletotrichum* from *Zea Mays* was 17-32 × 4-6μ and for *Sorghum vulgar Colletotrichum* 19-31 ×4-6μ.

VIII. Conclusion and summary

A disease of *Zea Mays* caused by a species of *Colletotrichum* was observed at Pusa. The fungus was isolated and its morphology, parasitism, cultural characters together with the effect of environmental factors on sporulation and on the germination of spores were studied.

Inoculation experiments carried out have shown that the organism can attack all the parts of the plant—leaves, midribs and leaf-sheaths. Cross-inoculation experiments carried out on a large number of allied plants showed that the parasite has a wide range of hosts under laboratory conditions.

Studies of the parasite in culture reveal that it grows better on natural than on synthetic media. Sporulation is favoured by high humidity and by alternate

light and darkness. Acervuli with setae are not frequently formed in culture. When the fungus sporulates poorly, appressoria are formed in great abundance either in contact with the hard surface of the glass container or in the substance of the medium.

It was noticed that high humidity, the presence of sugar, or the presence of living plant tissues accelerate the germination of spores. The optimum temperature for the germination of spores was found to be between 25°C and 30°C.

The fungus was compared with *C. graminicolum* isolated from *Sorghum vulgare*. The *Zea Mays* fungus can infect *Sorghum vulgare* and *vice versa*. The cultural characters and spore measurements show that both are very closely allied and thus the conclusion is drawn that the *Colletotrichum* sp. from *Zea Mays* is identical with *C. graminicolum* (Ces.) Wils.

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REDUCTION OF NITRATES BY THE SUN

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Berthelot and Gaudechon [1911] found that ultra-violet light induced the reduction of potassium nitrate in solution. The reduction was more in alkaline than in neutral or acidic solutions. Further in very weak solutions the reduction was practically complete.

Corbet [1934] using sunlight instead of ultra-violet light found that sunlight had a similar action though in a lesser degree.

The percentage of ultra-violet radiations in a beam of sunlight is small, yet it is an important source of ultra-violet light especially in tropical countries where the intensity of the sun is great. Therefore preliminary studies on the reduction of nitrates and the conditions affecting it were undertaken.

Experimental.—Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in each of the twelve Pyrex conical flasks of 300 c.c. capacity. Six of these were wrapped in black paper. These along with the rest were exposed to the sun. Estimations of nitrous nitrogen were made by the usual Griess Illosvay calorimetric method. The reduction is shown in Table I.

TABLE I

Showing the reduction of potassium nitrate

Time (days)	Mg. nitrous nitrogen per 100 c.c.	
	Unexposed	Exposed
1	*Nil*	0·039
	,,	0·036
2	,,	0·068
	,,	0·064
3	,,	0·091
	,,	0·091

For comparison the solution of the same strength was exposed to ultra-violet light from mercury arc lamp consuming about 1·6 amp. The solution was put in open Petri dishes and was placed directly one foot below the burner. There was considerable rise of temperature during the exposure. Representative samples for analysis were withdrawn after ten, twenty, thirty and forty minutes.

TABLE II

Showing the effect of ultra-violet radiations on the reduction of potassium nitrate

No.	Exposure (minutes)	Mg. nitrous nitrogen per 100 c.c.	Mean
1	10	0·110 0·123 0·104	0·112
2	20	0·220 0·233 0·229	0·227
3	30	0·311 0·362 0·337	0·337
4	40	0·427 0·518 0·440	0·461

The results given in Table II showed that ultra-violet light was a powerful reducing agent as compared to the sunlight.

Since ultra-violet light is absorbed by glass, the reduction of the substrate contained in Pyrex glass flasks and open Petri dishes of the same diameter was studied. Fifty c.c. of potassium nitrate solution containing one mg. nitrogen per c.c. was put in each of the containers and exposed to the sun. Loss of moisture was made up every half an hour. The contents were analysed after eight hours

TABLE III

Showing the reduction of potassium nitrate in flasks and Petri dishes

Container	Mg. nitrous nitrogen per c.c.	Average
Flasks	0·039 0·042 0·039 0·032	0·038
Petri dishes	0·045 0·064 0·045 0·054	0·058

The results (Table III) showed that the efficiency of the solar radiations was reduced by about 30 per cent by passage through Pyrex glass.

The nitrates of calcium, sodium, potassium, ammonium, magnesium aluminium, manganese and iron were similarly reduced when exposed to the sun.

The reduction of potassium nitrate was further studied with regard to the following :—

1. Concentration.
2. Temperature.
3. Season.
4. Influence of other substances.

1. Effect of concentration

Potassium nitrate solution (in 50 c.c. lots) of different concentrations was exposed to the sun in 300 c.c. conical flasks for forty-eight hours.

TABLE IV

Showing the effect of concentration on the reduction of potassium nitrate

Serial No.	Mgs. nitrogen per 100 c.c.	Mg. nitrous nitrogen	Percentage reduced
1	1·0	0·007	0·7
2	2·0	0·013	0·65
3	5·0	0·024	0·48
4	10·0	0·032	0·32
5	20·0	0·048	0·24
6	50·0	0·088	0·18
7	100·0	0·169	0·17

It is evident from Table IV that the percentage of reduction decreases with increasing concentration.

2. Influence of temperature

Three different temperatures, *viz.*, 10°, 25° and 45°C. were tried. Hundred c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in each of the six 300 c.c. conical flasks. These were placed in duplicates in three white enamelled trays containing water at different temperatures and exposed to the sun. Five-c.c. samples were withdrawn at ten minutes interval and treated with 0·5 c.c. of the reagent, put in $\frac{1}{2}$-in. cell and compared with standard slides.

TABLE V

Showing the effect of temperature on the reduction of potassium nitrate

Exposure (minutes)	10°C.±1·5	25°C±0·5	45°C.±1·5
10	1·1	1·4	1·6
20	1·6	1·9	2·2
30	2·0	2·2	2·6
40	2·2	2·5	3·3
50	2·4	3·1	3·5
60	2·6	3·3	4·0

The result given in Table V showed that the reduction of potassium nitrate increased with increasing temperature.

3. Effect of season

The reduction of potassium nitrate solution (one mg. nitrogen per c.c.) in fifty c.c. portions was studied during different months.

TABLE VI

Period of exposure (days)	Mg. nitrous nitrogen per 100 c.c.		
	January	May	September
1	0·016 0·015	0·045 0·045	0·039 0·036
2	0·024 0·023	0·089 0·065	0·068 0·064
3	0·032 0·029	....	0·091 0·091

The results (Table VI) showed that the reduction during summer was about thrice as much as in winter.

4 (a) Reduction of potassium nitrate in the presence of reduced nickel

Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in six 300 c.c. conical flasks. Reduced nickel (0·5 grm.) was added to four of them. All the flasks were placed in the sun ; two of them with nickel being wrapped in black paper.

TABLE VII

Treatment	Condition	Mg. nitrous nitrogen per 100 c.c. solution after			Percentage reduced
		1 day	2 days	8 days	
With nickel .	Unexposed	*Nil*	*Nil*	*Nil*	..
,,	,,	,,	,,	,,	
Without nickel .	Exposed .	0·036	0·068	0·356	0·332
	,,	0·032	0·062	0·308	
With nickel .	,,	0·071	0·156	0 972	0·988
,,	,,	0·068	0·169	1·004	

The results in Table V showed that the reduction was more in the presence of reduced nickel, than without it.

4 (b) Reduction of potassium nitrate in the presence of carbohydrates

Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in each of twenty-four conical flasks of 300 c.c. capacity. These were divided into six sets and each set was treated as shown below :—

1. No treatment.
2. 0·5 grm. sucrose per flask.
3. 0·5 grm. fructose per flask.
4. 0·5 grm. dextrose per flask.
5. 0·5 grm. maltose per flask.
6. 0·5 grm. potato starch per flask.

The flasks were plugged with cotton wool. Two flasks in each set were wrapped in black paper, and these along with the rest were kept in the sun. The contents of the flasks were analysed after an exposure of seven hours.

TABLE VIII

Showing the reduction of potassium nitrate in the presence of carbohydrates

Serial No.	Carbohydrates	Mg. nitrous nitrogen per 100 c.c.
1	Control	0·032 0·030
2	Sucrose	0·075 0·075
3	Fructose	0·075 0·071
4	Dextrose	0·078 0·084
5	Maltose	0·068 0·068
6	Starch	0·052 0·052

The results (Table VIII) showed that the reduction was more in the presence of carbohydrates. Starch gave the lowest figures, while it was practically the same in the case of other carbohydrates. Nitrous nitrogen in the flasks wrapped in black paper was only in traces.

The reaction is obviously a photo-chemical one, as the reduction in the dark was negligible. It is possible that the action of these substances may be a catalytic one.

4 (c) Influence of zinc oxide

Since zinc oxide is a powerful oxidising catalyst, it was interesting to see its effect on the reduction of potassium nitrate. It was found out that the reduction was completely stopped in its presence.

4 (d) Reduction of nitrates in the presence of soil

Soil has been found to act as a photo-catalyst for the oxidation of nitrogenous compounds [Sarkaria and Fazal-ud-Din, 1933]. The reduction of nitrates in its presence was also studied.

Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was put in each of the eight conical flasks of 300 c.c. capacity. Five grms. of soil were put in each flask. These were plugged with cotton wool and sterilized. Paper was tied over the plugs to protect them from dust. Four flasks were covered with black paper. These along with the rest were kept in the sun. A similar series with another soil was set up side by side. The contents of the flasks were analysed after twenty-five days.

TABLE IX

Showing the reduction of nitrate solution in presence of soil

Soil taken	Mg. nitrous nitrogen per 100 c.c.		Percentage reduced
	Exposed	Unexposed	
Loam	0·745	0·005	0·68
	0·606	0·006	
	0·713	0·007	
	0·680	0·006	
Clay loam	0·606	0·011	
	0·745	0·012	0·69
	0·713	0·012	
	0·745	0·013	

The results given in Table IX showed that nitrates were reduced in the presence of soil.

In order to find out the influence of soil on nitrate reduction, the experiment described above was re-started with varying amounts of soil (five and twenty grams) and four control flasks without soil. The contents of the flasks were analysed after twenty-five days.

TABLE X

Showing the effect of soil on the reduction of potassium nitrate in solution

(Mg. nitrous nitrogen per 100 c.c.)

Serial No.	Without soil	Loam		Clay loam	
		5·0 grms.	20·0 grms.	5·0 grms.	20·0 grms.
1	0·713	0·743	0·741	0·603	0·639
2	0·713	0·604	0·708	0·742	0·769
3	0·713	0·711	0·741	0·710	0·597
4	0·648	0·678	0·601	0·742	0·769
Average	0·697	0·684	0·698	0·699	0·693

The results (Table X) showed that the soils had no influence on reduction. It seems that the catalytic action of the soil is very feeble. Although, it is an oxidising catalyst, reduction took place in its presence without being influenced in any way by it, while in the presence of zinc oxide, potassium nitrate could not be reduced by the sun. It is obvious that the rate of photo-chemical reduction in the presence of soil is considerably greater than the rate of oxidation of nitrite to nitrate.

REDUCTION OF NITRATES IN SOIL

Two soils were moistened with potassium nitrate solution just below the sticky point. The nitrate solution in each case was of such a strength as to supply 100 mgs. of nitrogen per 100 grms. of soil. Fifty grams soil (on dry basis) was put in each of the eight 300 c.c. conical flasks. These were plugged with cotton wool and sterilized. Paper was tied over plugs to protect them from dust. Four flasks of each soil were wrapped in black paper and these along with the rest were exposed to the sun. Loss of moisture was not made up. The soils looked quite dry after a week. Nitrous nitrogen was estimated after twenty-five days.

TABLE XI

Showing the reduction of nitrates in soil

Soil taken	Mg. nitrous nitrogen per 100 c.c.		Percentage reduced
	Exposed	Unexposed	
Loam	0·047 0·054 0·039 0·050	0·021 0·027 0·019 0·019	0·026
Clay Loam	0·047 0·054 0·039 0·050	0·023 0·023 0·021 0·027	0·024

The results given in Table XI showed that nitrates were appreciably reduced in soil.

Nitrite formation in the exposed flasks may either be attributed to the oxidation of nitrogenous compounds of the soil or to the reduction of nitrates. But on exposing soils without potassium nitrate under identical conditions, no nitrite was obtained, therefore the excess of nitrite in the exposed flasks was due to the reducing action of the sun.

The results of reduction in soils, when treated statistically, gave the value of *S*, *S.E.* and '*t*' to be 0·00479, 0·00239 and 10·8 for loam soil and 0·00558, 0·00279 and 8·5 for clay loam soil. The expected value of '*t*' at one per cent from Fisher's table is only 5·487. Hence the results are significant.

The experiment was repeated with a smaller dose of potassium nitrate (ten mgs. nitrogen per 100 grms. of soil) and the results confirmed.

The above experiments seem to indicate that photo-reduction of nitrates in well-drained soils is of no importance. In case of water-logging the nitrates may partly be reduced photo-chemically.

EFFECT OF MOISTURE

Ordinary field soil was supplied with 100 mgs. of nitrogen as potassium nitrate per 100 grms. of air-dry soil. It was distributed in fifty-gram portions in twelve 300 c.c. conical flasks.

The soil in four flasks was kept in air-dry state. Out of the rest eight flasks, four were kept at thirteen per cent and four at thirty-nine per cent moisture. The flasks were closed with rubber bungs and sterilized. Analysis was done after eight days' exposure.

TABLE XII

Showing nitrate reduction in soil under different moisture conditions

Serial No.	Mg. nitrous nitrogen per 100 grms. soil		
	Air-dry	13 per cent moisture	39 per cent moisture
1	0·044	0·073	0·073
2	0·044	0·074	0·083
3	0·044	0·068	0·068
4	0·044	0·073	0·074
Average .	0·044	0·072	0·074

Initial nitrous nitrogen 0·039 mg.

The results given in Table XII showed that in dry soil the reduction was negligible. At thirteen per cent to thirty-nine per cent moisture it was appreciable and was un-influenced by moisture contents.

In order to see whether the effect of moisture was significant or not, the value of '*t*' was calculated. The results are highly significant as the calculated value of '*t*' is 21·7, while the value of '*t*' at one per cent from Fisher's tables is only 5·487.

EFFECT OF FARMYARD MANURE

Two per cent fermented cow dung was added to a portion of the soil and was thoroughly mixed. Five-gram portions of the manure and unmanured soil were added separately in flasks, containing fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) and sterilized. Four flasks, two of each lot were wrapped in black paper and these along with the rest were exposed to the sun and were analysed after twenty-two days' exposure.

TABLE XIII

Showing the effect of farmyard manure on the reduction of nitrates in solution

Soil treatment	Mg. nitrous nitrogen per 100 c. c.		Difference
	Exposed	Unexposed	
Manured	0·713 0·680	0·018 0·018	0·678
Unmanured	0·583 0·583	0.008 0.008	0·575

The results given in Table XIII showed that farmyard manure slightly increased reduction in nitrate solution.

The control soil flasks with fifty c.c. of distilled water were also exposed side by side with the other flasks to see if there was any nitrite production due to the oxidation of the organic matter, which was found to be nil.

The reduction was also tried in manured and unmanured soil, and was found to be practically the same in both.

Effect of soil packing

A soil with thirteen per cent moisture and 100 mg. nitrogen as potassium nitrate per 100 grms. of air-dry soil was distributed in eight conical flasks in fifty-gram portions on air-dry basis. The soil in four flasks was well pressed with a glass rod with a flattened end, while in the other flasks it was simply tapped to level the surface. The flasks were closed with rubber bungs and exposed to the sun after sterilization. The analysis was done after eight days.

Table XIV

Showing the effect of packing on the reduction in soil

Serial No.	Mg. nitrous nitrogen per 100 grms. soil		Mean difference
	Loose	Packed	
1	0·087	0·068	0·017
2	0·087	0·068	
3	0·078	0·068	
4	0·078	0·058	

The results given in Table XIV showed that the reduction was slightly more in loose packed than in hard-packed soil.

The penetration of light and surface area were less in compact soil than in the loose packed soil, hence the reduction was less in compact than in loose soil. The calculated value of 't' in this case is 4·89 while the expected value of 't' at two per cent from Fisher's table is 4·54. Hence the results are significant.

Conclusions

1. Nitrates of sodium, potassium, calcium, magnesium, manganese, ammonium, aluminium and iron were all reduced by the sun.

2. The lower the concentration, the higher was the percentage of reduction.

3. The nitrate reduction increased with the rise in temperature.

4. Reduced nickel, sucrose, fructose, dextrose, maltose and starch helped nitrate reduction.

5. The reduction of potassium nitrate in solution was not influenced in any way by the presence of soil.

6. The reduction was uniform within a wide range of moisture.

7. The reduction of nitrates in manured and unmanured soil was found to be practically the same.

8. The reduction was slightly more in a loose packed soil than in hard packed soil.

9. Photo-reduction of nitrates does not seem to be of any importance in soils.

Acknowledgments

The writer wishes to thank Dr. S. V. Desai, the Agricultural Bacteriologist, and S. Ram Singh Sarkaria for helpful suggestions during the course of the work.

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INHERITANCE OF SOME MORPHOLOGICAL CHARACTERS IN *CREPIS DIOSCORIDIS**

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INTRODUCTION

Since 1920 when Babcock pointed out the advantages of the genus *Crepis*, comprising about 200 species, for genetic investigations, considerable work has been done by members of the Division of Genetics of the University of California. Perhaps the most outstanding work on the inheritance of morphological characters in Crepis has been done by Rau [1923] and Collins [1924]. Rau [1923] reported investigations on the inheritance of three quantitative characters in *Crepis capillaris* (L.) Walb., *viz.*, length of leaves, number of lobes per leaf, and diameter of flower heads. He found that the length of leaf and the number of lobes per leaf, are heritable characters, but whereas environment greatly influences the expression of the former, it has little influence on the latter. At least four pairs of genes seem to be involved in conditioning the number of lobes per leaf. He selected two races of *Crepis capillaris* with different diameters of capitula and on crossing found that the diameter of the capitula of F_1 was intermediate between those of the two parents; no F_2 data were reported. He found moisture to be the limiting factor in determining the flower size.

Collins [1924] reported on the inheritance in *C. capillaris* of nineteen morphological and three physiological characters. The same author working with *Crepis dioscoridis* in 1926 found a plant which was wholly devoid of anthocyanin, and when crossed with the common pigmented type, the F_1 was fully pigmented. The F_2 cultures, segregated as follows : 103 purple to 38 green plants, clearly a simple 3 : 1 ratio. He concluded that one main gene conditions the production of any pigment in *C. dioscoridis*. Haney [1930] noted that yellowish green foliage colour in *C. dioscoridis* behaved as a simple recessive to normal green but no data was presented.

The present investigation deals with the inheritance of the following pairs of characters : presence or absence of anthocyanin, green or yellow foliage colour and normal or bushy habit of growth. The work was carried on partly in the greenhouse and partly in the field and the data combined.

*The investigation herein reported is a part of the thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Genetics in the Graduate Division of the University of California.

Material and methods

Seeds were germinated in sterile sand in two-inch earthen pots. The seeds were lightly covered and the pots placed in a shallow pan nearly full of water. Thus the sand absorbed enough water for germination of seeds. The majority of the seedlings emerged after four days and were planted singly in four-inch pots. Notes regarding the colour of the plants, whether green or yellow, were taken at the time of transplanting. The plants homozygous for the "yellow" gene may be recognized shortly after the seedlings appear, usually one day after, but notes were not taken until transplanting time so that the full quantity of chlorophyll peculiar to the respective individuals might develop.

Then, after about a month the plants were transplanted into six-inch pots. After another month the plants were set in the field where they could have the full sun. Normal *C. dioscoridis* is quite heavily pigmented with anthocyanin but in some cases the pigmentation is quite dilute and un-uniformly distributed. So, in determining the pigmented and non-pigmented types, plants should be grown in full light. Under such conditions pigment will be developed uniformly in the basal portion of flower stalks, except in those plants homozygous for the recessive condition. The two types can usually be distinguished in the early seedling stage if the seedlings are exposed to light and moderately low temperature. In such cases, the hypocotyl becomes pink in plants capable of producing anthocyanin and remains white or green in those lacking this ability. However, to avoid possible mistakes in classification of the two types, notes were completed when the plants had been about a month in the field.

Plants from culture 29·108 were intended for the determination of habit of growth whether normal or bushy and were, therefore, not set out in the field, because condition of the soil in the field may be very heterogeneous and uniformity in the environmental conditions is most desirable for the accurate determination of this character. So the plants were left in the greenhouse in the six-inch pots with an equal amount of potting soil in each pot.

In presenting data from hybrid populations, the degree of correspondence of observed with calculated distributions has been determined by the use of tables of probable error of Mendelian ratios prepared by the Department of Plant Breeding of Cornell University. In testing goodness of fit of dihybrid ratios, the method suggested by Harris [1912] has been used.

Brief description of cultures used

F_1 Culture—28·25= (26·1631-38 × 26·1740-69).

26·1631-38 Tall, anthocyanin absent, hairy, marginal achenes white, herbage green.

26·1740-69 Tall, anthocyanin present, glabrous, marginal achenes white, herbage yellow.

Six plants were isolated in this culture and all were tall, with anthocyanin present, and herbage normal green.

Plant 1—glabrous, marginal achenes dark or slightly whitened on ventral side.
Plant 2—hairy, marginal achenes white.
Plant 3—glabrous, marginal achenes white.
Plant 4—glabrous, marginal achenes dark or slightly whitened on ventral side.
Plant 5—hairy, marginal achenes white (occasionally one dark).
Plant 6—glabrous, marginal achenes dark or slightly whitened on ventral side.

These plants were grown together so that they may have inter-crossed among themselves.

F_1 Culture—29·108=(26·1455-3 × 26·1742-5)

26·1455-3 Tall, anthocyanin present, hairy, marginal achenes white, herbage normal green.

26·1742-5 Bushy, anthocyanin present, hairy, marginal achenes white, herbage normal green.

Seven plants were isolated from culture 29.108. All were tall, with anthocyanin present and hairy. Plant 6 was the only one with dark marginal achenes. For growing the F_2 population a small portion of seed from the progeny of each plant was used.

Experimental results

Inheritance of anthocyanin

Anthocyanin is found in many parts of the plant but is most noticeable in the midribs of the leaves and on the lower portions of the stems. The degree of its development may vary with different plants. In the plants with anthocyanin present the ligules usually have a red stripe on the back. Table I shows the data on segregation of hybrid plants.

Table I

Monohybrid segregation of non-pigmented plants from the progeny of pigmented plants

Culture 28·25	Total No. of plants	No. of plants with anthocyanin	No. of plants with no anthocyanin	Calculated		Deviations
Plant 1 . . .	31	26	7	24·75	8·25	1·25±1·63
Plant 2 . . .	83	63	20	62·25	20·75	0·75±2·66
Plant 3 . . .	66	53	13	49·50	16·5	3·50±2·37
Plant 4 . . .	95	72	23	71·25	23·78	0·75±2·85
Plant 5 . . .	62	46	16	46·50	15·5	0·50±2·30
Plant 6 . . .	98	77	21	73·50	24·5	3·50±2·89
Total .	435	337	98	326·25	108·75	10·75±6·09

The ratio in this case is 337 pigmented (red) to 98 non-pigmented (not red) plants and the deviation is less than twice the probable error. This is clearly a uni-factorial ratio, therefore this character is conditioned by a single dominant gene difference.

That this factor pair (**Aa**) controlling the production of anthocyanin is separately inherited from (**Gg**) a factor pair for chlorotic foliage colour or " yellow " is indicated by the F_2 distribution shown in Table II.

TABLE II

Dihybrid segregation in the progeny of self-fertilized F_1 plants (**AaGg**) *of culture 28·25*

	Total No. of plants	No. of red-green (A-G) plants	No. of red-yellow (A-g) plants	No. of non-red green (a-G) plants	No. of non-red yellow (a-g) plants	Deviation
Observed .	431	252	83	75	21	$\chi^2 = 4.48$
Calculated 9 : 3 : 3 : 1	..	242·4375	70·8125	70·8125	26.9375	($P = 0·2174$)

The goodness of fit value in this case is about twenty-one per cent which clearly indicates that these two factors are independently inherited. By using Immer's method for calculating linkage intensities [Immer, 1930] the ratio of product value is 0·8501 and the crossing-over value about forty-eight per cent which also indicates no linkage between these two factors.

Inheritance of " yellow "

" Yellow " (**gg**) is a recessive condition characterized by dilution of the quantity of chlorophyll and tentatively called " yellow " from the yellowish green colour as compared with the dark full-green pigmentation normal to the species. " Yellow " plants are usually a little smaller, grow more slowly, and mature later than the normals. Individual plants vary in degree of yellow colour, that is, some are deeper yellow than the others. It is quite possible that these differences may be merely due to the differences in nutrition.

The data are presented in Table III.

TABLE III

Monohybrid segregation of " yellow " plants from the progeny of F_1 green plants

Culture 28·25	Total No. of plants	No. of green plants	No. of yellow plants	Calculated 3 : 1		Deviation from 3 : 1
Plant 1 .	32	25	7	24·00	8·00	1·00 ± 1·65
Plant 2 .	85	62	23	63·75	21·25	1·75 ± 2·69
Plant 3 .	69	52	17	51·75	17·25	0·25 ± 2·43
Plant 4 .	100	74	26	75·00	25·00	1·00 ± 2·92
Plant 5 .	64	50	14	48·00	16·00	2·00 ± 2·34
Plant 6 .	103	79	24	77·25	25·75	1·75 ± 2·96
Total .	453	342	111	339·75	113·25	2·25 ± 6·22

The ratio in this case is 342 green to 111 yellow plants and the deviation is less than one third of the probable error. Hence " yellow " is a simple recessive to normal green. It has also been shown above (Table II) that factor pair (**Gg**) for yellow plant colour is not linked with the factor pair (**Aa**) which controls the production of anthocyanin in plants.

Inheritance of Bushiness

The bushy plants are characterised by having slender stems as well as a greater number of thin branches. They have a spreading habit of growth. There is no difference between the normal and the bushy when the plants are young but in the normal plants the flowering stalk becomes longer and the plants when mature can be easily separated.

TABLE IV

Segregation of bushy plants in the progeny of F_1 normal plants

Culture	Total No. of plants	Observed		Calculated		Deviation from 3 : 1
		Normal	Bushy	Normal	Bushy	
29·103	92	68	24	69	23	1·00 ± 2·80

The ratio in this case is sixty-eight normal to twenty-four bushy plants and the deviation less than one half of the probable error. This is a simple mono-factorial ratio ; therefore the character bushy must be conditioned by a single gene recessive to a gene responsible for the normal habit of growth.

GENERAL DISCUSSION

Unfortunately individual isolations of F_1 plants were not practised in both the cultures, therefore, no reliable data could be obtained on the colour of marginal achenes and hairiness *vs.* baldness in plants. At least plants with white marginal achenes should have been separated from those having coloured marginal achenes. In view of the fact that parent plants with white marginal achenes when crossed together gave an F_1 with coloured marginal achenes and the segregations in three of the F_2 families fitted so closely to 9 coloured : 7 white ratio, it is suggested that two complementary genes might be involved in the production of coloured marginal achenes. The occurrence of white marginal achenes in some of the F_1 plants shows that one of the parent plants was segregating for the complementary factor.

The objective in this work was to secure a strain of *C. dioscoridis* homozygous for all the recessive characters. There are four pairs of chromosomes in this species and it is desirable that each chromosome be differentiated by at least one gene effecting an easily distinguishable morphological character, in order that a further analysis of the species be made possible.

The studies of the comparative genetics of Drosophila and of rodents have proved that related species have germinal system which are either identical or very similar in organization. Collins [1924] crossed two glabrous plants of different races of *C. capillaris* from different localities and obtained all glabrous F_1 plants, proving that the same gene was responsible for the appearance of glabrous in each race. By crossing a non-hairy plant of *Crepis dioscoridis* with a non-hairy plant of *C. capillaris*, it can easily be demonstrated whether or not the recessive non-hairy is a case of parallel variation. The other recessive characters, anthocyanin absent and herbage yellow, which occur in this species, are also reported by Collins, to occur in *C. capillaris*. The occurrence of coloured marginal achenes is reported by Navashin [1929] in *C. aspera*. Thus there is evidence suggesting genetic parallelism in *C. capillaris*, *C. aspera* and *C. dioscoridis*. A parallel study of genetics and cytology of the three species and their hybrids might throw considerable light on their relationships.

The writer is indebted to Prof. E. B. Babcock at whose suggestion the investigation was started, and to Prof. R. E. Clausen and Dr. C. F. Pool for valuable help and suggestions during the progress of the investigation. Mr. C. W. Haney, Laboratory Assistant in the Division of Genetics kindly made the crosses and grew the F_1 plants.

Summary and conclusions

1. In the species *Crepis dioscoridis* three pairs of factors *viz.*, **Aa, Gg** and **Bb** are found to be responsible for the inheritance of the characters, anthocyanin present or absent, yellow or green plant, and bushy or normal habit of growth respectively, and probably two pairs of complementary genes are involved in the production of coloured marginal achenes.

2. Plants of *Crepis dioscoridis* are largely cross-fertilized.

3. *C. dioscoridis* and *C. capillaris* exhibit similar variations in morphological characters and it is possible that they may be closely related to each other.

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A NOTE ON A NEW TYPE OF PROGRESSIVE DAMAGE TO THE STRUCTURE OF COTTON HAIR CAUSED BY MICRO-ORGANISMS

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(With Plates L and LI and one text-figure)

The ultimate transformation of cellulose matter into carbon dioxide and water through the agency of micro-organisms is an important balancing process of nature. The manner in which this process is sometimes associated with structural changes in cotton hairs appears to be hitherto un-recorded. Denham [1922] states, " Actual damage may take place either from the organism attacking the wall and reducing its effective thickness *at one or more points** or from bursting of the wall by the pressure produced by internal growth ". It follows that Denham's description and illustrations refer to reduction in wall-thickness at one or more points only. At these points, apparently, the micro-organisms gradually drill holes in the fibre and damage its structure. Complete disappearance of secondary cellulose in a certain length of the fibre leaving an almost empty cuticular tube is neither described nor illustrated by any previous worker in the available literature.

Burns [1927] recognizes two types of organismal deterioration, *viz.*, (1) Deterioration due to actual decomposition ; and (2) deterioration due to the presence and growth within the cotton hair of micro-organisms, which may develop to such an extent as to affect the physical properties of the hair by simple growth pressure. Although the deterioration described below may appear to fall in type 1 as above, yet, again specific instances of this nature are not given either in his description or the photomicrographs. Thaysen and Bunker [1927] and Prindle [1934], who have made a detailed study of the micro-biology of cotton hair and in whose publications full references to earlier work will be found, also do not make any mention of the type of damage described here.

In the course of examination by Bright's Congo red technique of Broach Palej cotton for damage due to storage, a new type of deterioration in which the cell-wall is reduced to cuticular thickness over varying lengths in different fibres, was

* The *italics* are mine.

noticed. The normal thickness of the cell-wall gradually tapers towards the hollowed portion as shown in the camera lucida drawing of one such fibre below. A remarkable feature, therefore, of this type of damage is that the nature of maturity chracteristic, *i.e.*, the wall-thickness, is radically changed in the affected portions from that of mature or half-mature into immature type. In order to verify this observation, actual measurements of wall-thickness and ribbon-width of ten fibres in the normal and corroded parts of each were made. These are given in Table I. It will be seen from the mean values that whereas in the case of normal portions the wall-thickness of these swollen hairs is nearly half the ribbon-width, it is less than 1/12th of the ribbon-width in affected portions.

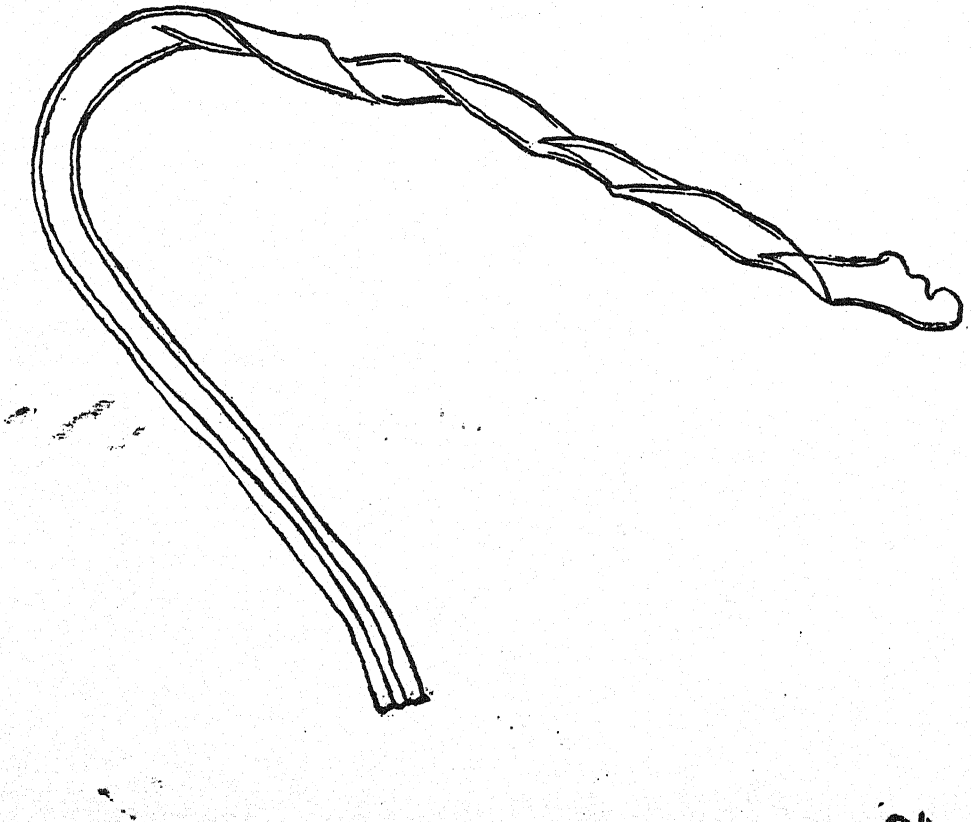

Fig. 1.

TABLE I

Fibre-number	Normal portion		Corroded portion	
	Ribbon-width	Wall-thickness	Ribbon-width	Wall-thickness
	Microns	Microns	Microns	Microns
1	32·71	13·75	35·31	2·61
2	24·88	12·32	26·68	1·90
3	36·02	17·06	32·23	2·37
4	23·70	11·61	25·36	2·13
5	18·49	7·58	18·49	2·37
6	26·31	9·24	33·42	3·56
7	23·70	9·71	28·44	3·79
8	22·51	10·67	23·46	1·42
9	30·81	9·48	31·05	1·90
10	29·62	10·67	34·60	2·13
Total .	268·75	112·09	289·04	24·18
Mean .	26·87	11·21	28·90	2·42

This damage is wholly internal in contrast to the two types already described which sooner or later lead to external injruy.

The original sample of this cotton before storage showed no instance of internal corrosion ; while the sample stored for six months showed about ten per cent of such hairs ; and after twelve months the corroded hairs were as numerous as twenty-five per cent. (In each case 300 fibres were examined from a representative sample). The increase in the number of affected hairs and also the affected length indicate that the change has been brought about during the period of storage.

The internal corrosion usually occurred towards the basal end of the fibre. In a few cases, however, it was noticed near the apical and also, when the latter happened to be broken. Again, instances of this hollowing process from the injured spot on either side of the injury were also observed. The maximum length affected in this manner extended up to one cm. from the broken end.

The reason why such damage occurred most commonly at the basal end is that the fibre is torn from the seed-surface, resulting in an opening in the lumen. This opening admits micro-organisms to the interior of the otherwise closed tube. Once the organisms enter the lumen, they not only obtain dried protoplasm as food material to thrive on, but also come in direct contact with the internally unprotected cellulose. The moment they establish themselves, they commence functioning as cellulose decomposers, if they happen to be of that type. They cannot do it as easily from the outside on account of the presence of a protective waxy layer of cuticle on the fibres.

Examination of numerous fibres (5,000) from end to end showed that the internal corrosion of cellulose is invariably associated with the presence of micro-organisms. Some seventeen species of fungi and three types of bacteria were isolated from this cotton. These will be described in a later publication. It is not easy at this stage to assign the internal corrosion to any one of these micro-organisms. Possibly both bacteria and fungi are involved in this process. The hollowed out parts were found to be localised near the broken ends or injury spots both of which are well-known seats of organismal infection. Furthermore, these parts were found to stain deep red by the Congo Red technique which according to Bright [1926] indicate moderate fungal damage. This, however, may be due to the action of bacteria, as Bright's technique is unable to distinguish between fungal and bacterial damage. The absence of secondary cellulose near the basal end of mature hairs is inexplicable on any other ground except as a result of the action of micro-organisms. Any interruption in the flow of protoplasm which is responsible for the deposit of cellulose in layers must essentially affect the rest of the fibre, as the source of nutrition is below its base. The fibres in question were, however, found to be quite well developed above the affected lengths. An alternative explanation on the basis of shrinkage of cellulose but not of cuticule may even be considered. This is also contrary to the observation of Osborne [1935] that cuticle shrinks in length on being treated with caustic soda and in circumference on being washed in water after the process of mercerisation. The cell-wall was usually found to taper towards the hollow portions in which lay disintegrated layers of cellulose wall. In the event of mere shrinkage there is no reason for the cell-wall to taper or lose its compactness. It is, therefore, concluded that internal corrosion of cotton fibre is wholly attributable to the decomposing action of micro-organisms.

In support of the above view nine photo-micrographs are reproduced below. Plate L, fig. 1 shows the basal end of an undamaged hair. Plate L, fig. 2 shows the beginning of the internal corrosion which extends up to the point marked by the letter A. In Plate L, fig. 3 the damage has progressed to about 0·7 mm. Fig. 4 shows a still greater length affected in this manner, the letter A again marking the extent to which corrosion has progressed. Plate L, fig. 4 shows corrosion on either side of injury at B occurring about the middle of a fibre. Plate L, fig. 5 brings out clearly that the cell-wall tapers from the normal to the corroded region. Plate LI, fig. 1 shows the details of the affected part. The presence of some spirillar organisms as indicated by the arrow may be noted. Plate LI, fig. 2 illustrates organisms in the lumen of an otherwise normal portion of cotton fibre. This fibre looks highly magnified as it was flattened out by pressure in the course of examination. Plate LI, fig. 3 relates to two fibres, a thin one and a broad one, the latter showing, in its interior, dislocated layers of cell-wall, which precede corrosion at the transition point between the normal and the affected regions. Plate LI, fig. 4A shows partly peeled off layers from the cell-wall, while 4B shows some of the organisms, at a high magnification, in the corroded region.

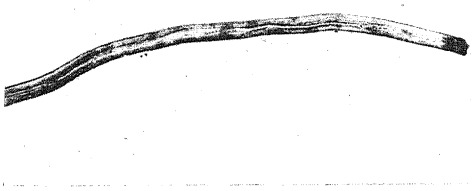

FIG. 1 (×100)

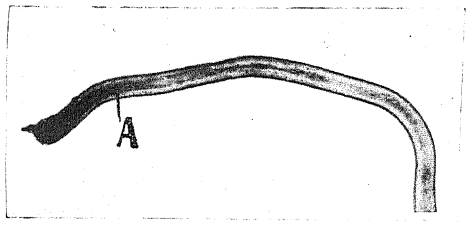

FIG. 2 (×100)

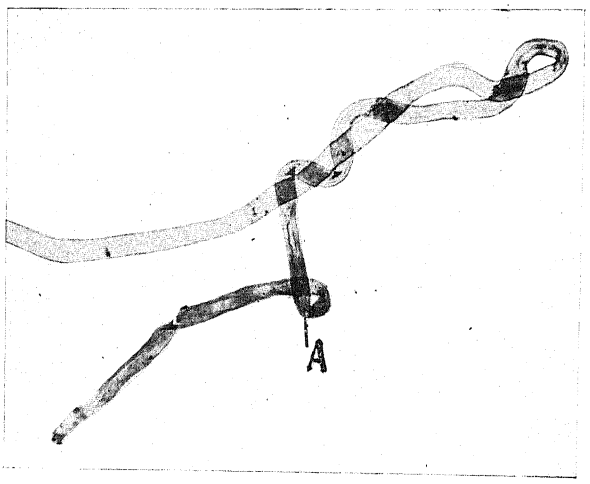

FIG. 3 (×100)

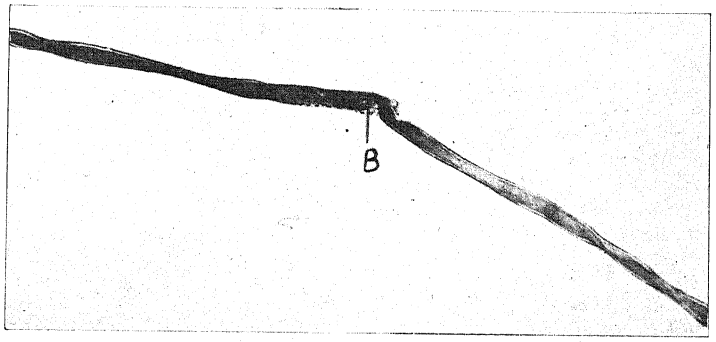

FIG. 4 (×100)

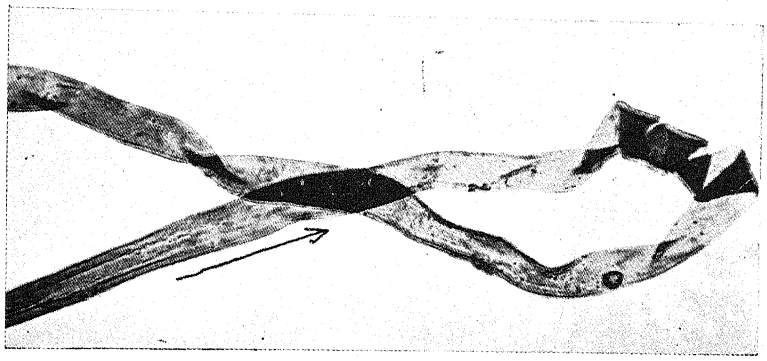

FIG. 5 (×200)

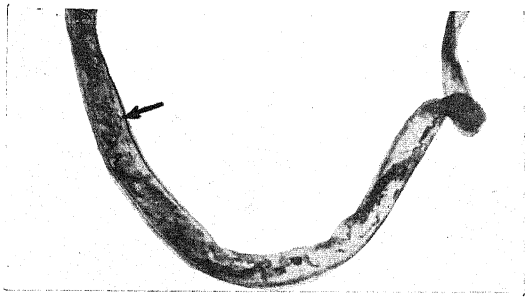

FIG. 1 (×180)

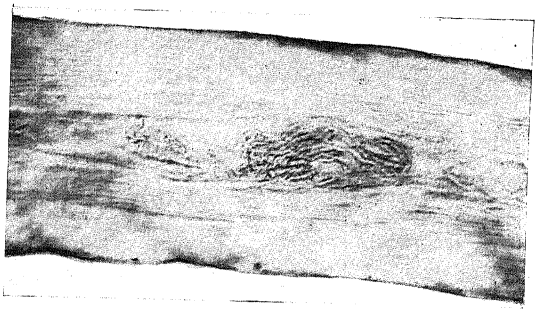

FIG. 2 (×325)

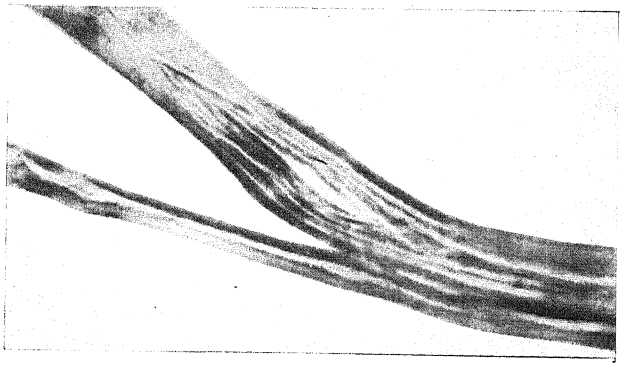

FIG. 3 (×325)

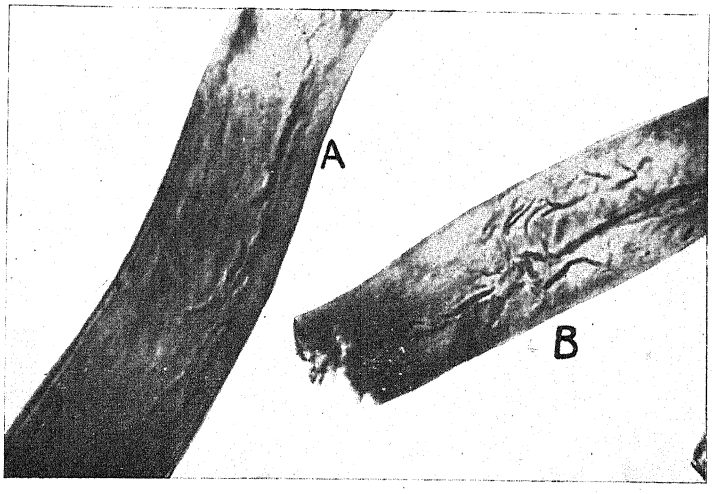

FIG. 4 (×700)

In

FIG

SUMMARY

While investigating the causes of deterioration of Broach cotton, a type of damage to the structure of cotton fibre was discovered which does not appear to have been described by the earlier workers. The chief characteristics of this damage are that (1) it commences from the usual seat of organismal infection, *i.e.*, the basal end or a tear in any other part of the fibre ; and (2) it slowly progresses internally disintegrating and corroding the cell-wall, leaving intact merely cuticular sheath. The cell-wall gradually tappers from the unaffected portion into the affected one. Nine photo-micrographs are reproduced in support of the observation.

In the end the writer takes this opportunity of expressing his gratitude to Dr. Nazir Ahmad, Director of the Laboratory for his helfpul guidance.

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SELECTED ARTICLE

METHODS OF ANALYSIS AND APPRECIATION OF WHEAT, FLOURS AND BREAD, PARTICULARLY IN RESPECT OF INVESTIGATIONS ON THE BAKING VALUE

BY

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(With Plate LII)

FOREWORD

The numerous researches recently made in regard to the chemistry of cereals by well-known scientists and investigators throughout the whole world have led to considerable changes in analytical chemistry and have shown the necessity for bringing up to date and unifying the methods of analysis and research. Ing. Arnaldo Luraschi, President of the "Federazione Nazionale dei Panificatori", made this necessity clear at the 1st International Bread-making Congress (Rome, 1932) and his proposals were again vigorously defended at the 3rd International Technical and Chemical Congress of Agricultural Industries (Paris, 1934) and at the 16th International Agricultural Congress (Budapest, 1934).

It is certain that great improvements would be effected in the cereal trade if it were possible to come to an international agreement on the processes and methods of analysis and estimation. This, however, is a very delicate and complex question which is more difficult to solve than would appear at first sight. In fact, it is not only a question of standardising present methods, but it is above all essential that analysts and investigators should put their own personal feelings on one side and work together with the same methodical and scientific spirit.

The object of this present article is merely to present and to specify the most recent and accurate methods of research in the hope that an international meeting of scientists, technicians and persons interested in the question will be held in the near future and will lead to an agreement, and also to a practical application as indicated above.

To return to the subject, according to the object of the research the method of analysis may be classified in three categories :

(1) analysis of wheat ;
(2) analysis of flours ;
(3) experimental tests and analysis of bread.

It is certain that, with regard to the baking value, the most satisfactory determination is obtained by the practical proof of baking. In fact, the quality of the bread obtained allows an indirect, but exact, estimate to be made of the baking " strength " of the flour.

It must, however, be added that analytical research is always necessary as not only does it demonstrate the various chemical, physical and physico-chemical properties of flours and the origin of certain phenomena, but also may provide elements whereby it will be possible rapidly to appreciate the qualities of the product examined.

In this first chapter research on wheat will be discussed and in the following chapters flours and bread.

Chapter I

Analysis of wheats

Wheats have very variable characteristics and properties, dependent on numerous factors which are connected not only with the variety, but also with the systems of cultivation, the nature of the soil, climate, etc.

There are a great number of varieties of wheat, several tens of thousands, and the number increases continually owing to the work of selection and hybridisation.

Following the production and trade classification wheats may be placed in three large categories :—

(1) Hard wheats (*Triticum durum* Desf.) with hard and heavy grain, compact and flinty on breaking.

To this category belong the hard wheats of strong bread-making quality of the types Manitoba, Bankuti, etc., and also the wheats with yellow, golden or straw coloured grain chiefly suitable for the manufacture of alimentary pastes. These latter are grown principally in hot countries.

(2) Semi-soft wheats (*Triticum turgidum* L.), average sized grains, yellowish in colour, on breaking starchy in the centre and flinty on the outside. In Italy these wheats are grown in the central regions.

(3) Soft wheats (*Triticum vulgare* Willd.) differentiated from the preceding wheats by the fact that the grains break readily and are white and starchy throughout.

As has already been seen, not all wheats are suitable for bread-making ; while many soft and semi-hard wheats have the characteristic qualities for the preparation of light and well-risen bread, a certain number of hard wheats, particularly suitable for the manufacture of alimentary pastes, have properties which render them unsuitable for bread-making.

A flour, to be really suitable for bread-making, should have a combined set of characters which are known in the trade as the " strength of the flour ". This is the combination of chemical, biochemical, physical and physico-chemical

properties which, on the one hand, give a normal and adequate fermentation and, on the other, impart to the dough a sufficient resistance to the pressure of gases disengaged as to confine them as much as possible. Hence the mass dilates and its volume expands uniformly and a bread is obtained which is large-volumed, light, appetising and perfect in all respects.

Wheats and flours are known as "strong" when they possess the properties indicated to an adequate degree while the "weak" wheats and flours are characterised by an insufficiency of these properties.

Passing on the analysis of wheat, a few brief words will now be said on the principal determinations, at the same time particularly emphasising the latest improved methods.

The present methods of appreciating wheat may be classified under four headings :—

(1) Methods for determining physical and organoleptic characteristics.
(2) Methods of investigating the chemical properties.
(3) Methods based on biochemical determinations.
(4) Methods relating to the yield in flour.

It is, however, advisable to first say a few words on the taking of samples, grinding and preparation of the product.

Taking of samples.—The essential condition for obtaining accurate results in the analysis of cereals is to take samples which represent an average of the product examined. For this purpose suitable apparatus are introduced into the mass of grain and several samples are taken from various depths. These are then mixed together into the shape of a square. This square is divided into four parts by the two diagonals. Two of these parts, the opposite angles of which touch, are then taken. If the sample is too large the above process is repeated until a sample is obtained suitable for research purposes.

Grinding and preparation of the product.—Before being subjected to chemical analysis, the wheat should be ground so that it constitutes a fine and homogeneous mass, uniform and constant in composition. For this purpose a small laboratory mill is required and the grains should be ground in such a manner that the chemical composition of the product is not altered. This work must, therefore, be carried out slowly, avoiding any heating of the flour which would affect the composition. The flour thus obtained is conserved in glass pots hermitically sealed with parafin until such time as they are utilised for further investigation.

A. The study of the organoleptic and physical properties of wheat, which are of great importance from the scientific and practical point of view, relate to the following characters :—

(1) Purity of the wheat ;
(2) Weight of 1,000 grains:
(3) Volume of 1,000 grains ;

(4) Weight per hectolitre ;
(5) Specific weight ;
(6) Flintiness or hardness.

Determination of purity.—The purity of the wheat is a valuable factor for the classification of the wheat from the standpoint of commercial value. It would be desirable to be able to fix a maximum tolerance of the total quantity of foreign bodies (2 per cent for examples), as these foreign bodies are the cause of considerable loss during the processes of cleaning, especially if they are in the form of particles of iron, small stones, etc.

The purity of the wheat is determined in a very simple manner ; after having taken a sample of 100 grammes of wheat, all the foreign substances are separated from the mass by means of a magnifying glass and a pair of small pincers. The sample is then weighed again and brought up to 100 grammes.

Weight of 1,000 grains.—The weight, which is in direct relation to the size of the grains, is generally determined by means of a special grain-counting apparatus.

Volume of 1,000 grains.—In the majority of cases there is a direct relation between the volume of the grains and the yield in flour. The volume of 1,000 grains is determined by means of a small pycnometre fitted with a graduated tube. The apparatus is filled with xylol, 1,000 grains are introduced and the volume may be immediately read off from the graduated tube. The data obtained are very satisfactory from the practical point of view. If more accurate results are required, a volumenometre must be used which will be mentioned again later when the specific weight is discussed.

Weight per hectolitre.—Special importance is attached to this point in the purchase and sale of wheat although it does not precisely indicate the true milling yield. It should be noted here that the true commercial value of wheat is to be estimated not only according to the yield in flour, but also according to the use to which it will be put. It has in fact been ascertained that the milling value of wheats is shown opproximately by the weight per hectolitre, while there is no relation between this weight and the suitability of the wheat for bread making. Several factors influence the value of the weight per hectolitre, among which are the following :—

(1) The space occupied by the wheat which is dependent on the close packing between one grain and another.
(2) The specific weight.

These factors, in their turn, are dependent on : the chemical composition of the grain, and especially on its content in water and bran ; the volume ; the shape ; the presence of foreign bodies and imperfect and unripe grains ; the smoothness of the surface of the grain on which depends the slipping down or heaping up of the mass. It is understood also that the variation in the weight per hectolitre is also influenced by the soil and climatic conditions of the place of origin of

the crop, by the degree of ripeness, or of dryness, by the purity, etc. To these factors, which are dependent on the quality of the product, must be added other factors related to the methods employed for warehousing the wheat. The weight of the hectolitre varies considerably according to the height and capacity of the warehouse and the quantity stored, and it is thus that, according to different methods of storing, (sacks transported and emptied by hand, " transporter " elevators, suction elevators, etc.) the heaping of the grain will vary and with it the weight per hectolitre.

In the trade it is more important to obtain values which are comparable and correspond to each other than very accurate results; also it is essential strictly to follow the prescribed rules and to employ the methods and apparatus officially adopted. The Schopper apparatus with a capacity of one litre is the most utilised; special tables give the weight of the hectolitre calculated from the figures shown on the scale. It is advisable for this determination to place the wheat in the apparatus by means of a filler of funnel shape with a wide base so as to obtain the same packing each time*.

The weight of wheat to the hectolitre varies from 68 to 84 kg. and is as much as from 87 to 88 kg. for hard wheat.

Specific weight.—The value of this weight varies from 1·28 to 1·50 and is in strict relation to the quantity of the cereal examined.

In the opinion of certain authors, the specific weight of a wheat should always be determined in order to appreciate the commercial value as this would be much more accurate than the weight per hectolitre the determination of which is influenced by various factors.

The specific weight varies according to the differences in the nature of the product examined and, with regard to cereals, two factors must be taken into consideration :—

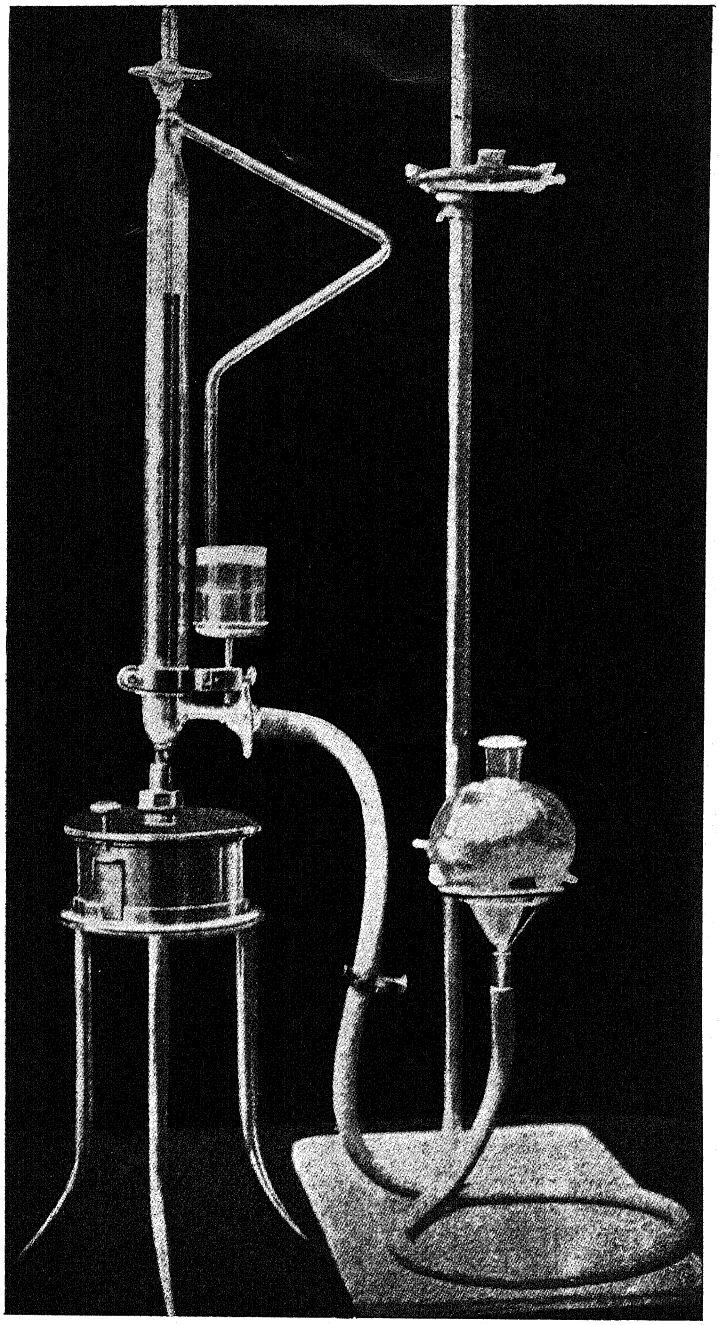
(1) The air content ;

(2) The chemical composition of the cereal.

Air is present either in the external integuments or in the albumen ; the specific weight diminishes as the air content increases. Humidity greatly influences the specific weight, also the protein substances, there being a diminution in the specific weight in proportion to an increase in these substances.

For determining the specific weight of cereals, the apparatus used at present are ordinary pycnometres or special pycnometres (Bruckner-Kuhn apparatus) and the method of heavy liquids of Prof. Neumann is followed (Zeitschrift fur das gesamte Muuhlewesen, 1927,4). These processes are not, however, free from error as the xylol, as well as the other liquids, does not adhere perfectly to the surface of the grain and is also more or less absorbed by the grain.

* E. Schmidt has invented a new appratus whereby by means of a system of small gratings the packing of the wheat in the receptacle is simplified and is more uniform and constant. The results are higher, but more in agreement and more accurate (Das Mihlenlaboratorium, 1932. 4).



Volumenometre with direct reading.

The most accurate method, and the one that should be adopted for cereals, is certainly that which is based on the use of volumenometres ordinarily employed in physics for determining the specific weight of substances with a high absorbent capacity.

In our laboratory we have used a volumenometre invented by our collaborator, Dr. De Rege, which has the advantage of giving a direct reading. The characteristic of this apparatus is that the glass tube containing mercury is graduated so as to correspond to a given barometric depression. The calculation is very rapid. The apparatus is calibrated, the recipient is filled with a certain quantity of grain and the result is read and verified by means of the manometre. The total specific weight is obtained by dividing the number of cubic centimetres occupied by the cereal by the weight of the cereal.

Specific weight of various wheats

Wheats	Pycnometre		Bruckner Kuhn apparatus (Xylol)	Method of Prof. Neumann	Volumeno-metre	Weight per hectolitre (in hg.)
	Water	Xylol				
Amber	1,250	1,378	1,377	1,384	1,410	82·15
Senatore Cappelli	1,224	1,335	1,334	1,360	1,385	78·60
Manitoba	1,256	1,355	1,356	1,382	1,415	83·70
Mentana	1,248	1,402	1,400	1,385	1,419	80·35
Balilla	1,249	1,355	1,352	1,374	1,410	80·35
Virgilio	1,234	1,355	1,355	1,375	1,416	78·60
Catria	1,258	1,332	1,330	1,371	1,399	78·80
Zara	1,230	1,355	1,350	1,375	1,400	81·23
Fausto Sestini	1,257	1,333	1,330	1,375	1,420	76·80
Damiano Chiesa	1,268	1,334	1,333	1,372	1,400	81·25

The data given by the volumenometre are higher, but more accurate. It must also be remarked that there is no strict relation between the value of the specific weight and the weight per hectolitre.

Determination of flintiness.—According to a great number of authors, the degree of flintiness or hardness is in direct relation to the quantity of nitrogenous substances and gluten contained in the wheat. The wheats richest in flinty grains are the most sought after for making alimentary pastes, while those richest in starchy grains are most suitable for manufacturing starch. It must be remarked that there are hard wheats which are not suitable for making pastes, while being eminently suitable for bread-making.

For the purpose of determining the hardness of wheats, special apparatus for taking sections are used and the percentage of hard, semi-hard and starchy grains is calculated.

B. Chemical analysis of wheat generally includes the following determinations :—

(*a*) Determination of humidity,

(*b*) Determination of ash,

(*c*) Determination of proteic substances,

(*d*) Determination of fats,

(*e*) Determination of crude cellulose.

Determination of humidity.—Humidity is an element of great importance in estimating the value of wheat from the commercial standpoint and from that of its keeping qualities. The humidity content of wheat is chiefly dependent on the climatic conditions in which it has been grown. Humidity appears under various forms, especially :

Absorption humidity ;

Vegetation humidity ;

Humidity of chemical constitution.

The first is derived from the soil, the second is due to rains, mists, dew, etc.

Vegetation humidity is present in all the phenomena of development, growth and maturation of the plant. The cereal loses a part in drying and retains a quantity varying from ten to sixteen per cent.

As to the third type of humidity, constituted by the water of crystallisation and that derived from the disintegration of molecules, no investigation has been made.

Several methods are followed for determining the humidity. In our laboratory the process is as follows :—

Five grms. of wheat flour are taken and placed in a calibrated weighing bottle, it is then dried in a stove at 105°, up to constant weight (six hours)*.

Determination of ash.—The ash content in wheats varies from 1·5 to 2·6 per cent and the wheats poorest in ash are generally the least rich in cellulose.

For the determination of ash the process is as follows :—Five g. of wheat flour is carefully weighed in a platinum or porcelain evaporating dish and then

* The determination of humidity in a stove at 105° does not give very accurate results and as a rule there are errors of about 0·3 per cent. To eliminate completely the last traces of humidity a Liebig U-tube must be used, brought up to 105° and through which a current of dry air or inert gas is passed. The rapid methods recommended lately such as those based on the use of high temperatures, or on the determination of water by distillation in the presence of special liquids, or on the determination of humidity by electric constants, although of great interest on account of their rapidity, do not give an absolutely accurate determination.

placed in a muffle furnace which is progressively heated up to a maximum temperature of 650°. For rapid results it is advisable to moisten the mass after carbonisation with small quantitites of oxidising substances such as neutral oxygenated water, a watery solution of ammonium carbonate or nitrate, etc.

Determination of nitrogenous substances.—The content of wheat in nitrogenous substances is in direct relation to the gluten in the flour and varies greatly from one wheat to another. With certain hard wheats it is as much as nineteen per cent.

The quantity of nitrogen is ascertained by the Kjeldahl-Ulsch method, using 3 gm. of flour, 25 ccm³ of phospho-sulphuric acid (125 gm. phosphoric anhydride dissolved in 1,000 ccm³ of sulphuric acid at 66°B), a small quantity of copper oxide (0·05 gm.) and four to five drops of a ten per cent solution of platinum chloride. The quantity of protein substances is obtained by multiplying the nitrogen content ascertained by the factor 6·25. The American official method prescribes the factor 5·7, while the figure 6·25 is generally adopted in European laboratories.

Determination of fats.—Ten gm. of flour are placed in an extraction filter and dried for two hours in a hot water oven. Extraction is then carried out for ten hours in a De Soxhlet apparatus with anhydrous ether. The filter is removed and the balloon flask containing the ether extract is heated to eliminate the ether. The final traces of ether are removed by heating the open balloon flask in a bain-marie. The residue is then dried for one hour in a hot-water oven and then weighed. The fat content varies greatly in different wheats, and may be from 1·5 to 1·9 per cent.

Determination of crude cellulose.—For this operation the Weende method is the most advisable. The procedure is as follows :—

In a porcelain evaporating dish is placed 3 gm. of flour with 200 cm³. of sulphuric acid (1·25 per cent) and boiled for 1½ hours, adding water as evaporation takes place. It is then filtered and the contents of the filter is collected in the evaporating dish by a jet of water ; 200 cm³ of water is added and the whole is boiled for half an hour. It is then filtered again, the water treatment is repeated ; it is boiled again and the residue is placed in the filter.

The residue in the filter is boiled again in the same evaporating dish for half an hour with 200 cm³ of a solution of caustic potash (1·25 per cent) and filtered. The water treatment is repeated twice, boiling each time for half an hour.

All residue not dissolved is passed through a calibrated filter, previously dried at 105°, it is first washed in boiling water then cold water, and finally in a mixture of alcohol and ether, it is then dried at 105° and weighed. The filter and its contents are placed in a crucible, the whole is incinerated and weighed. The quantity of crude cellulose, referred to 100 parts of substance, is obtained by deducting from the weight first obtained the weight of the ash. The proportion of crude cellulose varies from 1·5 to 3 per cent and even 4 per cent for certain wheats.

C. Bio-chemical tests, which are made on the whole wheat, include all the methods whereby the baking value of the variety may be determined with more or less accuracy.

The need for a method suitable for the requirements of farmers and geneticists (who, for the creation of new varieties, must carry out tests on small quantities of wheat) has resulted in new studies being made by numerous investigators.

The method most widely used by technicians is certainly that of Dr. Pelsehnke of Halle University. (Archiv fur Pflanzenbau, 1930, 5). This scientist has introduced a bio-chemical process called "Schrogarmethods" for determining the quality of wheat from the point of view of bread-making. The process is as follows :—

Ten g. of whole flour is kneaded with 0·5 gm. of yeast and 6 cm^3 of water so as to obtain a dough of average consistency. Contrary to what usually occurs with ordinary flour, whole flour, according to the writer, will always have the same degree of water absorption, so that the tests will give constant results.

The dough is divided into two equal parts and well kneaded by hand on a glass sheet so as to form two balls of dough, taking care to make them perfectly homogeneous. These two balls are placed in two glasses 6 cm. in diametre and 7 cm. in height, and filled with 75 cm^3. of water. These are placed in a thermostat at 32°. The time when the balls are placed in the glasses is noted and also the moment when, after the splitting of the dough into fragments these fall to the bottom of the glass. The time, in minutes, which elapses between the beginning of fermentation and the break up of the dough is taken as an index figure of the quality of gluten. Weak flours break up rapidly, strong flours slowly.

These index figures vary greatly according to the variety of wheat. The finest wheats have indices exceeding eighty ; the weakest have indices below thirty.

This method, which according to numerous experimenters gives results very fairly in agreement with baking tests, is particularly valuable in the field of genetics with regard to the choice and estimation of new varieties.

Another rather similar method is the Guttingen (Das Muhlenlaboratorium, 1934, 7 and 10). The increase in volume of the ball of dough is determined during fermentation and consequently the capacity of the dough to retain gases. This property is more pronounced in the flours of superior quality.

D. Investigations on the milling yield.—An approximate appreciation of wheat from the point of view of milling is arrived at in the trade, on the basis of weight per hectolitre.

It is generally considered that wheats having a weight per hectolitre less than 80 give a yield in flour of less by 1 to their weight per hectolitre, while the wheats with a weight per hectolitre higher than 80 give a yield in flour of less by 2.

This naturally only serves as an indication and the desirable precision cannot be obtained in this way as other factors, such as humidity, size and shape of the grains, percentage of foreign bodies, etc., also have an influence.

Thanks to modern methods of milling, the miller obtains several qualities of flour from the wheat which are sold under different names according to the choice of the producer and the purposes for which they are intended.

With a view to judging the milling quality of a wheat, millers take as a basis the various qualities of flour obtained in identical conditions. It must be remarked that the yield in flour also depends, in addition to the quality of the wheat, on the system of milling and the improved character of the milling apparatus.

Investigators in the laboratories extract a single quality of flour known as whole or complete flour, obtained from wheat with the bran removed, extraction being effected in apparatus of reduced size and according to different methods.

It is certain that the results thus obtained cannot agree. In fact, scientific and practical methods must be followed which give results corresponding to and in agreement with those obtained at the mill.

For this reason we have adopted a small experimental roller mill called the "Vottero experiment mill" and with which we obtain very satisfactory result.

MYCOLOGICAL NOTE

ON THE SYSTEMATIC POSITION OF THE SMUT CAUSING MALFORMATION OF *VITIS QUADRANGULARIS*

BY

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(Received for publication on 21st February 1936)

In 1922 Iyengar and Narasimhan [1922] reported a smut from Madras on *Vitis quadrangularis* Wall., which caused a distinct witches' broom of the host. The disease started at the nodes where the diseased parts branched repeatedly and irregularly, bearing dark-green swellings which, when cut open, showed dry, black, powdery mass of spores. The spores were coupled in pairs and possessed a dark-brown, smooth epispore ; the authors were unable to induce spore-germination. The smut was described as a new species of *Schizonella* and given the name *S. colemani*.

The genus *Schizonella* was created by Schroeter in 1877 to describe a smut whose sori are formed in the leaves and whose spores occur in pairs, being joined together in the middle by a kind of isthmus. The spores form *agglutinated* masses and are *serially* produced in the fertile hyphae. Clinton [1904] states that both Schroeter and Brefeld had succeeded in germinating the spores of the species. The species of *Schizonella* so far described seem to be confined to temperate climate and to the family Cyperaceae.

Beck erected another genus, *Mycosyrinx*, to describe the tropical smuts with paired spores. The sori in the case of this genus occur in the interior of the pedicels and the peduncle causing a witches' broom and contain a *powdery* mass of spores. The spores are produced *singly* at the ends of the fertile hyphae and their germination has not so far been observed. The genus seems to be confined to the family Vitaceae and to the tropics. Only two species have so far been described : *Mycosyrinx cissi* (DC) Beck and *M. arabica* (Henn.) Penzig. The latter of these is reported on *Cissus quadrangularis* which is a synonym for *Vitis quadrangularis* Wall.

The writer has seen Iyengar and Narasimhan's type specimen (part of which, kindly given to him by Professor M. O. P. Iyengar, is in the Pusa Herbarium) and it is manifest that the part of *V. quadrangularis* that is affected is the peduncle. The spores are in a *powdery* mass and their measurements are in agreement with those given by Penzig. There seems to be no doubt that the smut belongs to the genus *Mycosyrinx* and not to *Schizonella*.

The species of *Mycosyrinx* (*M. arabica*) mentioned above as parasitic on *Vitis quadrangularis* is known to occur in India ; it was collected by Dr. W. McRae at Mettupalayam, Madras Presidency, in 1911 and was identified by Sydow [1914]. A herbarium specimen of the smut which exists at Pusa, has been compared with Iyengar and Narasimhan's specimen and the result of the comparison leaves no doubt that the smut named by them as *Schizonella colemani* is identical with *Mycosyrinx arabica* (Henn.) Penzig and that the former name should therefore be abandoned.

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NOTES

TABLES OF STANDARD ERRORS OF MENDELIAN RATIOS

Tables of Standard errors of Mendelian ratios have been prepared for the 1 : 1, 3 : 1, 9 : 7, 15 : 1, 13 : 3, 27 : 37 and 63 : 1 ratios, in the Botanical Section of the Imperial Institute of Agricultural Research, Pusa, by Dr. S. S. Purewal and Mr. P. Krishna Rao, and have been published as Miscellaneous Bulletin No. 11 of the Imperial Council of Agricultural Research.

These tables give directly the standard errors for values of n from 5 to 1,000, and can also be used for determining any value of n beyond 1,000 and up to 100,000 by moving the decimal point and by simple linear interpolation where necessary. The method of using the tables is simple and is explained in the introductory note which precedes them. They should prove of considerable use to plant breeders and geneticists.

B. P. P.

PROCLAMATION OF THE UNION OF SOUTH AFRICA GOVERNMENT

PROCLAMATION 290 OF 1930

* *No. 290, 1930*

Whereas Mosaic Disease of Sugar Cane, being a plant disease in terms of the Agricultural Pests Act, 1911 (Act No. 11 of 1911) has been found to exist in the areas enumerated in the Annexure hereto ;

And whereas it is deemed to be necessary to impose restrictions for the prevention of the spread of the disease ;

Now therefore under and by virtue of the powers in me vested by paragraph (*c*) of section fourteen of Act No. 11 of 1911, I do hereby declare, proclaim and make known that the prohibition and restrictions set out in the Annexure hereto shall exist in respect of the removal of the plants therein named, to the extent therein provided.

Any person guilty of a contravention of any such prohibition or restriction, or failure to comply with any provision of this Proclamation with which it is his duty to comply, will, under section twenty-seven of the said Act, be liable to the penalties in that section mentioned.

Proclamation No. 12 of 1927 is hereby repealed as and from the date of publication of this proclamation.

God Save the King.

Given under my Hand and the Great Seal of the Union of South Africa at Pretoria this Twenty-sixth day of November one thousand Nine hundred and Thirty.

ATHLONE,
Governor-General.

By Command of His Excellency the Governor-General-in-Council.

J. KEMP.

ANNEXURE

1. For the purpose of this Proclamation the following are declared to be restricted areas :—

The Province of Natal and Zululand, excluding the following Magisterial Districts of Northern Zululand:

Ubombo and Ingwavuma

2. From and after the date of publication, no person shall remove or cause to be removed, except by the special permission of an officer appointed by the Minister of Agriculture, any sugar cane plant or any part of sugar cane plants of any variety other than the varieties Uba, Ch 64/21, P. O. J. 2878, and P. O. J. 2725 from any place within the restricted area to any other place either within that area or outside thereof.

The expression " variety Uba " means any sugar cane plant which has the characters of the leaf and stalk of the cane recognized in Natal as Uba and has not been raised from seed cane derived from any plot or field which the Government Mycologist has certified to be wholly or in part infected with Mosaic disease.

The expression " variety Ch 64/21" means any sugar cane plant which has the characters of the leaf and stalk of the cane recognized by the Government Mycologist as Ch 64/21 and which has been derived from the seed cane which was imported from Cuba under the name of Ch 64/21 by the issue of the Plant Importation Permit No. 89 of 15th March, 1926.

The expression " variety P. O. J. 2878 " means any sugar cane plant which has the characters of the leaf and stalk of the cane recognized by the Government Mycologist as P. O. J. 2878 and which has been derived from seed canes which were imported from the Incomati Estates, Portuguese East Africa, under the name of P. O. J. 2878 by the issue of Plant Importation Permits Nos. D. 585 of 21st October 1927 and D 719 of 20th September 1928.

The expression " variety P. O. J. 2725 " means any sugar cane plant which has the characters of leaf and stalk of the cane recognized by the Government

Mycologist as P. O. J. 2725 and which has been derived from the seed canes imported from the Department of Agriculture, U. S. A. and from Incomati Estates, Portuguese East Africa, under the name of P. O. J. 2725 by the issue of Plant Importation Permits Nos. D 504 of 9th March, 1927, and D 585 of 22nd October 1927, respectively.

PROCLAMATION 290 OF 1930

Amendments to regulations

* *No. 2192* (*5th December 1930*)

For the purpose of dealing with the outbreak of Mosaic Disease in sugar cane in the areas restricted by Proclamation No. 290 of 1930 the Governor General has been pleased, in terms of section *twenty-eight* of the Agricultural Pests Act, 1911 (Act No. 11 of 1911) to make the following regulations :—

Regulations

1. The regulations published under Government Notice No. 64 of the 14th January, 1927, are hereby repealed as and from the date of the publication of the regulations hereunder.

2. For the purpose of these regulations the expressions " variety Uba " "variety CH 64/21 " " variety P. O. J. 2878 " and " variety P. O. J. 2725 " shall be as defined in the Annexure to Proclamation No. 290 of 1930.

3. From and after the date of publication of these regulations no person shall plant or cause to be planted in any area restricted by Proclamation No. 290 of 1930, which areas are set out in the schedule to these regulations, any sugarcane of a variety other than the varieties Uba, CH 64/21, P. O. J. 2878 and P. O. J. 2725 without a written permit from any officer appointed by the Minister of Agriculture.

4. From and after the date of publication of these regulations no person shall keep or cause to be kept in the said area any sugar-cane of a variety other than the varieties Uba, CH 64/21, P. O. J. 2878, and P. O. J. 2725 without a written permit from an officer appointed by the Minister of Agriculture.

5. Where under sub-section (2) of section *fifteen* of Act No. 11 of 1911, the Minister specially empowers an officer to take such steps as may be necessary for eradicating the disease on any premises, the said officer may require by fourteen days' notice the destruction of sugar-cane of any variety other than Uba and the destruction shall be carried out in the manner prescribed in the said notice.

Schedule

The following is the area restricted by Proclamation No. 290 of 1930 :—

The province of Natal and Zululand, excluding the Magisterial Districts of Ubombo and Ingwavuma.

* *No. 809* (*9th June, 1933*)

His Excellency the Governor-General has been pleased, in terms of section twenty-eight of the Agricultural Pests Act, No. 11 of 1911, to make the following

amendments to the regulations published under Government Notice No. 2192 of 1930, as amended by Government Notice No. 1364 of 1931.

A. By the cancellation of the amendments published under Government Notice No. 1364 of 1931 and the substitution of the following new amendments.

B. By the cancellation of paragraph 2 and the substitution of the following new paragraph :—

(2) For the purpose of these regulations, the expressions " Variety Uba," "Variety CH 64/21," " Variety P. O. J. 2878 ", Variety P. O. J. 2725 ", "P. O. J. 2727 " " P. O. J. 2714 ", " Co 290 " and " Co 281 " shall be as defined in the Annexure to Proclamation No. 290 of 1930 as amended by Proclamation No. 119 of 1933.

C. By the addition of the words " P. O. J. 2727 ", " P. O. J. 2714 ", " Co 290 ", " Co 281 " after the words "P. O. J. 2725 " where they appear in paragraphs 3 and 4.

EXTRACT FROM UNION GOVERNMENT GAZETTE No. 2299 OF 27TH SEPTEMBER 1935

* *No. 197, 1935.*

Under and by virtue of the powers in me vested by paragraph (*d*) of section *fourteen* of the Agricultural Pests Act, 1911 (Act No. 11 of 1911) as replaced by section two of the Agricultural Pests Amendment Act 1934 (Act No. 15 of 1934) I do hereby declare, proclaim and make known that Proclamation No. 290 of 1930, as amended by Proclamation No. 119 of 1933 is hereby further amended by the addition of the words " and also Co 301 " after the words " Co 281," where they appear in paragraph 2 of the Annexure to the said Proclamation No. 290 of 1930 as so amended.

The expression " Co 301 " is defined in the Annexure hereto.

God Save the King.

Given under my Hand and the Great Seal of the Union of South Africa at Pretoria this Twentieth day of September, One thousand Nine hundred and Thirty-five.

J. W. WESSELS,
Officer Administering the Government.

By Command of His Excellency the Officer Administering the Government-in-Council.
D. REITZ.

ANNEXURE

The expression " Co. 301 " means any sugar cane that has the characters of the leaf and stalk of the cane variety recognised by the Government Mycologist as Co 301 and that has been derived from the seed canes which were imported from the Imperial Sugar Cane Station, Coimbatore, South India, under the name of Co 301 by the issue of Plant Importation Permit No. D. 598 of the 18th October 1930.

REVIEW

Vernalization and phasic development of plants. *Bulletin No. 17 of the Imperial Bureau of Plant Genetics:* Herbage plants, and Occasional Publication of the Imperial Bureau of Plant Genetics for Crops other than Herbage: Joint Publication—Aberystwyth and Cambridge, Dec. 1935. (Price 10/-).

The idea of vernalization originated as a result of a new outlook on plant growth and development formulated by the Odessa school of plant physiologists headed by T. D. Lysenko. Until 1930, it was unknown to wider circles of agriculturists, though since that time it has assumed a very prominent place in agricultural literature and thought. The general principles of the theory are now widely familiar. These are roughly that growth and development, far from being identical as has been popularly supposed, are in reality two distinct and separate phenomena. Growth is regarded merely as increase in size and weight and such changes as flowering and reproduction are included under development. According to the new theory either of these two processes may proceed independently of the other, so that we may be faced with the extraordinary case of a plant which comes into flower without having grown or a plant which grows for ever without ever coming into flower: All that it is necessary to do it would seem, to produce such remarkable behaviour, is to treat the plant in such a way that one of the two processes is favoured to the exclusion of the other. If seeds of winter wheat are germinated in an ice box and subjected to suitable conditions of light, aeration, humidity and other factors, they can be sown in spring and will come into ear at the same time as if they had been sown in the ordinary way in autumn. Hence the term " vernalization," which is a Latinized equivalent of the Russian word " jarovizacija " coined by Lysenko to describe the phenomenon and which strictly means " transformation of winter forms into spring ".

The matter was first brought to the attention of English-speaking readers by a bulletin issued in 1933 by the Imperial Bureaux of Plant Genetics. This early bulletin gave a complete outline of the method as it was described in Lysenko's first publications, and consisted mainly in the description of a practical method for the pre-sowing treatment of seed to obtain this accelerated development in a variety of crops. The method was rapidly seen to be of practical importance not merely in Russia but in all parts of the world. In view of the wide interest taken in the subject the Imperial Bureaux have continued to collect the literature relating to it. This has assumed such magnitude and the subject has become so much extended in its range that the whole matter has been reviewed afresh in a new and much enlarged bulletin covering some 150 pages.

This new bulletin describes in detail the results of the many experiments that have been carried out on all sorts of crops under every possible condition, both in the Soviet Union and in other countries all over the world. The results of vernalization of over 6,000 varieties of wheat and of 500,000 hectares of vernalized sowings, based on replies to a questionnaire received from 1,056 different Soviet farms are reported. The results have on the whole been satisfactory when the necessary conditions of technique have been observed and it would seem that the method may now be definitely regarded as a useful agricultural measure in countries where early ripening is a decisive factor in successful cultivation. For instance, spinach, sugar beet and even potatoes have by suitable treatment been grown at Hibiny in the arctic circle. Cereals have been induced to ripen a week or more earlier and by this means it has become possible to extend their cultivation to large tracts of country where this has been impossible hitherto because of drought or other unfavourable conditions. The main results of the Russian workers have now been confirmed in most countries where vernalization has been tried. In countries of more normal climatic conditions the method will probably not be of such wide application to the main crops but in the case of salad, vegetable and market garden crops a difference of a few days may prove of tremendous economic importance and a considerable future is foreseen in the application of vernalization to plants of this type. The Russian workers have even claimed to get increased yields from vernalized sowings but there seems to be some difference of opinion on this point and others have observed a depression in yield after vernalization.

At least half the bulletin is devoted to the physiological aspect of the phenomenon of vernalization. Lysenko's original conception has been extended to cover the whole range of plant development, in the form of the theory of Phasic Development. Development, says Lysenko, consists of a series of stages, each of which requires for its completion a definite combination of external conditions. The conditions for one phase may be different from those required for others, temperature being the decisive factor for the first stage and light for the second. Each stage must be completed before the succeeding one can be initiated but once any stage has been completed there is nothing to prevent the progress of the following one, provided the necessary external conditions are forthcoming. The method of vernalization therefore consists in the provision at as early a point in the life of the plants as possible of the requisite conditions for all the developmental stages leading up to reproduction, thus curtailing the long periods of time that normally occur between these successive stages. A freshly germinated seed has been found to be the most suitable material to work on, as this can be conveniently treated before sowing and then sown in the ordinary way.

Other Soviet botanists, though agreeing with Lysenko's general findings, disagree with many of his conclusions and have put forward theories of their own. The views of these other workers are presented in the bulletin side by side with those of Lysenko in a spirit of pure impartiality. In fact a careful study of this

bulletin shews that it has dealt with the available information from all possible points of view, equally generous treatment being given to the practical application of the method in agriculture and horticulture and to its theoretical explanation. It will be of interest to plant physiologists, biochemists, plant breeders, teaching botanists and indeed to anyone concerned with modern trends of botanical and agricultural research. " The rapid development in scientific research is one of the outstanding features of the Twentieth Century," as Sir David Chadwick aptly remarks in his forward ; " Research workers need the earliest possible information of developments affecting their line of study but frequently find themselves baffled by its volume and by linguistic difficulties." To overcome these difficulties the Imperial Agricultural Bureaux were organized in 1929 and the bulletin under review affords an admirable example of how these Bureaux fulfil their function. The large majority of the articles with which the bulletin deals are written in Russian and would have remained entirely unknown to the rest of the world if they had not been made available in the present form. A total of over 200 separate articles are considered and reviewed in detail and large sections of the tabulated results of the authors are reproduced in a review of the work of the whole world on this controversial but entrancing new subject.

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6305-22 Botany.

6

ORIGINAL ARTICLES

STUDIES ON *BEMISIA GOSSYPIPERDA*, M. & L.

No. 3* SEASONAL ACTIVITIES OF *BEMISIA GOSSYPIPERDA*, M. & L. (THE WHITE-FLY OF COTTON) IN THE PUNJAB

BY

M. AFZAL HUSAIN, M.A. (CANTAB.),
Entomologist to Government, Punjab,

KIDAR NATH TREHAN, M.Sc.,
Assistant Cotton Entomologist,

AND

PIARE MOHAN VERMA, M.Sc.,
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(Received for publication on 3rd February 1936)

(With two text-figures)

CONTENTS

	PAGE
I. INTRODUCTORY	894
II. TECHNIQUE	894
III. MIGRATIONS OF THE PEST	894
1. Migration from cotton to the winter alternative host-plants	895
2. Migration from the winter alternative host-plants to the spring alternative host-plants	895
3. Migration from the spring alternative host-plants to cotton	896
IV. CROSS-INOCULATIONS	896
V. HOST PREFERENCE FOR OVIPOSITION	897
VI. PERIOD OF SEVEREST INFESTATION ON COTTON	898
VII. SUMMARY	899
REFERENCES	903

* No. 1. A Preliminary Note on the White-fly of Cotton in the Punjab. *Agric. J. Ind.*, Vol. XXV, Part VI, Nov. 1930.

No. 2. Observations on the Life-history, Bionomics and Control of the White-fly of Cotton (*Bemisia gossypiperda*, M. & L.). *Ind. J. Agric. Sci.* Vol. III, Part V, Oct. 1933.

I. Introductory

Since *B. gossypiperda* can obtain subsistance from a variety of plants, belonging to over thirteen different families [Husain and Trehan, 1933 ; Massop, 1932], it is obvious that any enquiry into the bionomics of this White-fly cannot be complete without an appreciation of its activities on its alternative hosts. Moreover, the intensity of infestation on cottons depends, besides other factors, on the survival of the pest during the season when the cotton crop is not growing, and also on its initial population at the beginning of the cotton season. Evidently, therefore, the possibility of an effective prevention by the ' safe disposal ' of the alternative host-plants cannot be ignored. Therefore, observations were made during 1932 and 1933 on the incidence of *B. gossypiperda* on its various host-plants, with a view to determine its preference for any of them and to discover the relative importance of the different hosts as sources of infection for the subsequent cotton crop.

The Indian Central Cotton Committee financed a scheme of research on the White-fly of cotton and this paper deals with a portion of the work done under this scheme. The generosity of the Indian Central Cotton Committee is gratefully acknowledged.

II. Technique

Entire plants, or where that was not practicable, entire leaves* of the various food-plants of *B. gossypiperda*, wild and cultivated, collected from within a radius of about two miles from the Agricultural Research Institute, Lyallpur, were examined and White-fly infestation worked out in relation to leaf area. During these investigations about 27,594 leaves of cottons with an area of 139,082 square inches, and 20,949 leaves of other host-plants, 69,339 square inches in area, were examined. For the study of host preference for oviposition, experiments were carried out in the laboratory in small muslin cages.

III. Migrations of the pest

Since the most important cultivated food-plants of *B. gossypiperda* are annuals, and the wild food-plants are mostly herbaceous perennials, the pest must of necessity live on its different hosts during different parts of the year. Irrespective of any preference for any of its food-plants, the density of White-fly population on a particular host is influenced to a large extent by its proximity to the cotton fields infested by the White-fly, protection from wind and adequate humidity. Another factor, perhaps of the greatest significance, is the stage of growth of the plant. Further, as stated by Nath and Suryanarayana [1929], Metcalf and Flint [1932], Delong [1934], Hargreaves [1934], Jackson [1934] and others, the radical changes in the composition of the cell sap in the leaves of the plants at different periods of growth, indicating the physiological state of the host, may be of the greatest importance. These changes may be responsible for bringing about seasonal migrations of *B. gossypiperda* from host to host.

* In case of such plants as cucurbits, a number of leaves could only be examined.

Whatever may be the cause of these fluctuations in population, this pest has been observed to undergo, in general, three phases of migration during the course of a year.

(1) Migration from cotton to the winter alternative host-plants

During November when the cotton crop is maturing and the leaves are drying up and shedding, the population of White-fly falls greatly on this plant, and its infestation starts on the alternative host-plants such as, rape (*Brassica napus*), cauliflowers (*Brassica oleracea*), turnips (*Brassica rapa*) and potatoes (*Solanum tuberosum*) among the cultivated plants, and *Sonchus* spp., *Euphorbia* spp., and *Convolvulus arvensis*, etc., among the commoner of the weeds.*

From December onward the number of adults falls considerably but the immature stages, *viz.*, eggs and nymphs, remain on these alternative hosts throughout the winter (Table 1).

It will not be out of place to mention that at times the intensity of infestation on certain of the host-plants increases sporadically. For instance, during November and December 1932 the intensity of attack on *Solanum tuberosum* and *Duranta plumeri* was very high and was much severer than on any of the other host-plants. Further, it has also been observed that the intensity of attack may vary, on the same host, growing in two different situations in the same locality, in which case, probably, the microclimatic factors play an important part.

The adults commence emerging from about the end of January and multiply once again on the winter host-plants such as *Brassica napus*, *Brassica oleracea*, *Brassica rapa*, *Raphanus sativus*, *Sonchus* and *Euphorbia* spp. and *Convolvulus arvensis*. The population thus increases for the future spread of the pest.

(2) Migration from the winter alternative host-plants to the spring alternative host-plants

By the end of March the various cultivated cucurbits, namely, *Cucumis melo*; *Citrullus vulgaris*; *Cucumis sativus*, *Lagenaria vulgaris* and *Luffa acutangula*, etc., come up and the White-fly migrates from the winter alternative food-plants to these spring hosts, where rapid multiplication takes place. Besides these, the infestation at this stage is also observed on *Nicotiana tabacum*, *Hibiscus esculantus* and *Althea rosea*, and from April onward the ratoon cottons are also severely infested. By the time the infestation has increased on the above-named host-plants, in general, the infestation on weeds has fallen considerably.

It has been observed, that, during April and May the ratoon cottons and the cucurbits, in general, and melons in particular, form the most important breeding

* The pest has also been recorded on various other plants but infestation on these is very light.

centres. Hence, the ratooning of cotton and growing of melons mixed with cotton, or close to cotton fields, is likely to lead to a severe White-fly attack to the next cotton crop.

(3) Migration from the spring alternative host-plants to the cottons

The White-fly makes its appearance on the new cottons early in May, that is, soon after the crop has germinated, but the attack at that stage is extremely low as compared to the other host-plants. From June, however, partially because of the migration from the alternative host-plants, but mostly on account of the rapid multiplication of the pest, the intensity of attack increases enormously on the new cotton crop. During this period the attack on the cucurbits declines, but continues to be high on the ratoon cottons uptil the middle of July. In 1932, both the new and ratoon cottons were allowed to grow together in the same plot and relative incidence of the White-fly attack was worked out from June to the end of August. During June and upto the 5th of July the infestation was higher on the ratoon cotton and after that it was higher on the new crop (Table below).

Relative incidence of White-fly attack on the ratoon and fresh cotton crop in 1932

Host-plants	Average number of immature stages of White-flies per square inch of leaf area									
	18-VI	26-VI	5-VII	13-VII	20-VII	30-VII	6-VIII	12-VIII	19-VIII	26-VIII
Ratoon 4 F cotton	6·8	6·8	9·2	10·2	16·4	9·6	5·7	3·1	2·1	...
New 4 F cotton	2·7	5·4	7·1	10·2	19·1	16·9	9·7	4·8	2·4	1·6

These observations lead us to the conclusion that cucurbits and ratoon cottons act as the immediate and direct sources of White-fly infestation for the new cotton crop. The winter crops and particularly the weeds which harbour the immature stages of this pest during the winter are also of very great importance. Hence the value of clean cultivation and destruction of weeds is quite obvious.

The seasonal history of the pest in relation to some of the more important host-plants has been tabulated in Table II.

IV. Cross-inoculations

To verify the status of the various hosts as the true food-plants of *B. gossypiperda*, series of cross-inoculations were tried.* For each of these experiments, adults of White-fly were collected from definite host-plants and liberated on to

*A few of these crosses had been tried previously and the results confirmed. *Ind. J. Agric. Sci.*, Vol. III, Part V, October 1933, p. 701-753.

the other host-plants, which had been grown under glass chimneys to keep them free of infestation. The following cross-inoculations have been tried successfully :—

Adults of *B. gossypiperda* collected from	Life-cycle completed on
Cotton	Cucurbits ; *Sonchus arvensis* ; *Achyranthus aspera*, *Brassica oleracea* ; *Digera arvensis* ; *Chaenopodium murrale* and *Raphanus sativus*.
Convolvulus arvensis . .	Cotton ; *Solanum nigrum* ; *Sonchus arvensis* ; *Brassica oleracea*.
Cucurbits	Cotton.
Althea rosea. . . .	Cotton.
Lycopersicum esculentum .	Cotton ; *Achyranthus aspera*.
Ipomea sp.	Cotton.
Brassica oleracea . .	Cotton ; *Withania somnifera* and *Brassica rapa*.
Solanum melongena . .	Cotton ; *Chaenopodium murrale*.
Solanum nigrum . . .	*Brassica oleracea*.
Sonchus arvensis . . .	*Brassica rapa*.
Achyranthus aspera . .	*Brassica rapa*.
Ratoon cotton . . .	*Raphanus sativus*.

These observations have shown that the plants cited above are the actual host-plants of the White-fly of cotton. Further, it has also been noticed that even *B. gossypiperda* is not so unorthodox as to feed on all and sundry plants. Repeated attempts were made to obtain eggs of this White-fly on leaves of *chari* (*Andropogon Sorghum*), but without success.

V. Host preference for oviposition

In order to study host preference, observations were made as to the relative oviposition on *Gossypium* spp., *Althea rosea*, *Lagenaria vulgaris*, *Cucumis melo*, *Luffa acutangula* and *Cucumis sativus* from May to July, and on *Gossypium* spp. *Brassica rapa*, *Raphanus sativus*, *Brassica oleracea*, *Solanum melongena*, *Brassica napus*, *Solanum tuberosum* and *Lycopersicum esculentum* from October to December, *i.e.*, corresponding to the periods of migration in nature from the spring alternative host-plants to cotton and from cotton to the winter host-plants respectively. The plants under observation were sown together in small beds and were enclosed, soon after germination, in small muslin cages, 2 ft. × 2 ft. × 1½ ft. For each observation about five hundred adults were collected in a glass chimney, which was then placed in the middle of the cage. The mouth of the chimney was covered over with a cardboard and the adults were allowed to escape through a small hole. After twenty-four to forty-eight hours' exposure, the plants were removed and all the leaves examined under a binocular and the eggs laid recorded. For comparison, the data were correlated with the leaf area examined. During May, relatively more eggs were laid on melons (*Cucumis melo*) than on cotton, but during July, oviposition increased considerably on cottons, var. Mollisoni

leading. From October onward, oviposition decreased on cotton and increased on *Brassica* spp., *Solanum tuberosum* and other alternative host-plants (Tables III and IV) and Fig. 1.

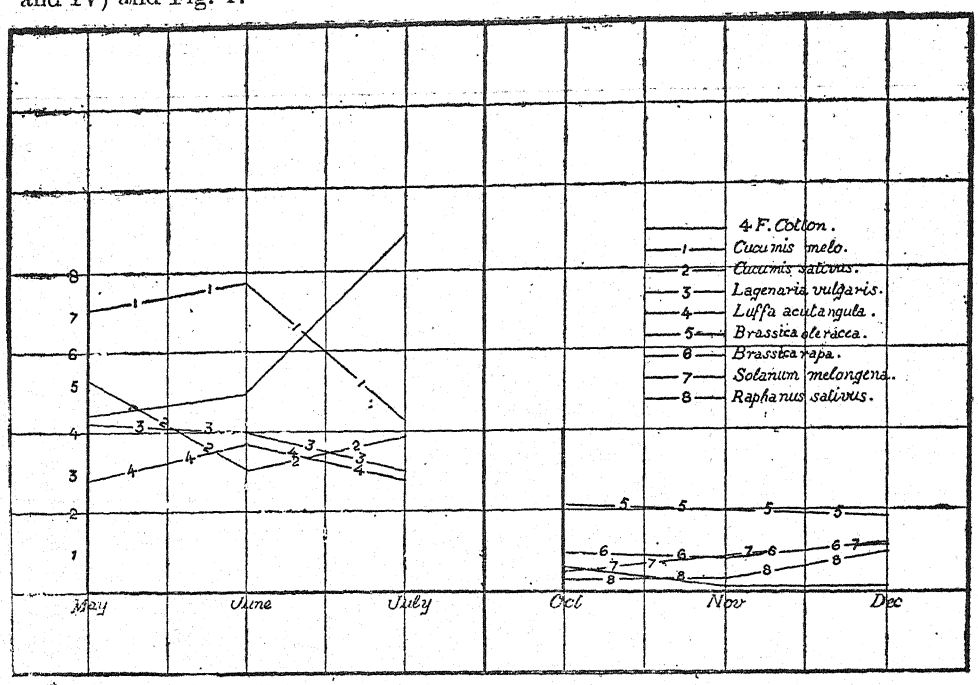

Fig. 1. Showing relative oviposition of *B. gossypiperda* on cotton and other alternative host-plants.

VI. Period of severest infestation on cotton

A study of the life-history of *B. gossypiperda* has shown that the duration of its life-cycle from April to September is, on an average, about eighteen days. The shortest duration of fourteen days was noticed during August, while the longest duration of eighty-eight days in the laboratory, extended from November to February. The most favourable period of quick multiplication of the pest, therefore, coincides with the period of germination and growth of the cotton crop.

To determine the severity of White-fly attack and relative infestation on different varieties of cotton, observations were continued from 1931 to 1933 on six varieties, namely, Mollisoni, Sanguinum and Rosea representing *desi*, and 4 F, 285 F and 289 F, representing the American types. For each observation all the leaves on individual plants of each type were examined and the White-fly infestation was worked out in terms of leaf area. Some striking differences have been observed among these varieties regarding the initial attack. To start with, the intensity of the White-fly attack is comparatively severer on the *desi* (indigenous) varieties—Mollisoni leading, but from about the middle of August the infestation increases relatively on the American varieties. During 1933, however, the intensity of

attack, in general, was considerably low, hence the difference in attack on the various varieties was not so marked. The data collected with regard to the relative infestation on Mollisoni and 4 F varieties are presented in Table V and Fig. 2. It has been established that the period of the severest attack on cottons extends from June to August, after which the infestation, as a rule, declines abruptly.

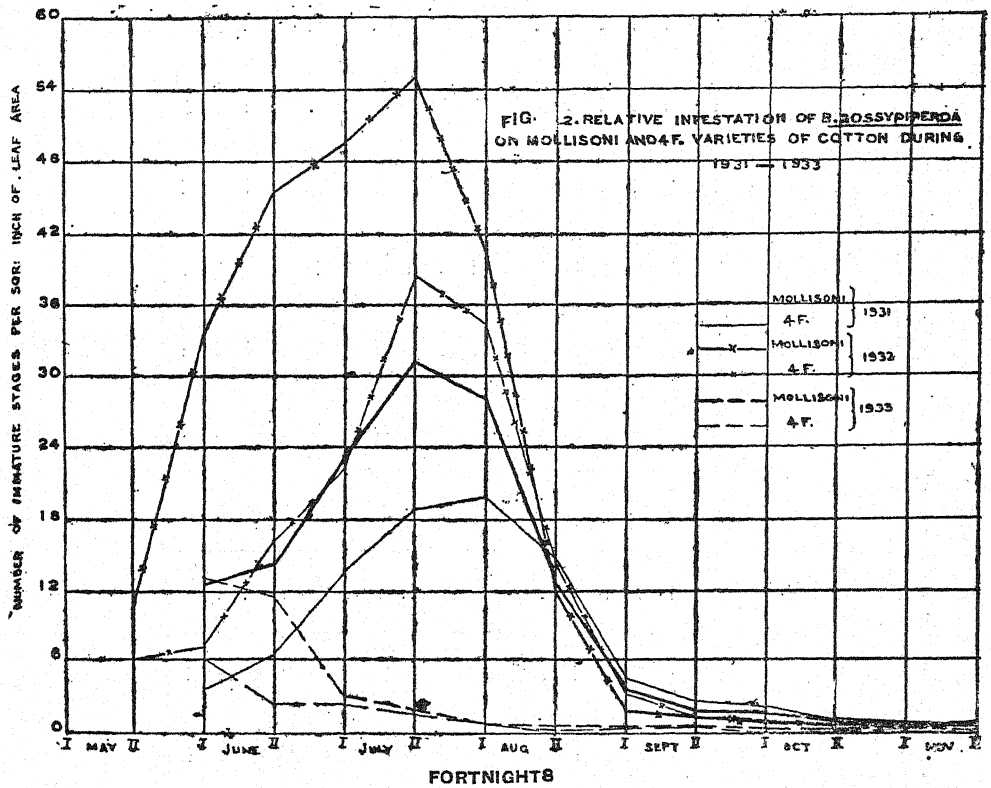

Fig. 2. Relative infestation of *B. gossypiperda* on Mollisoni and 4 F varieties of cotton during 1931-1933.

VII. Summary

A study of the seasonal activities of *B. gossypiperda* shows that there are three phases of migration of the pest in the course of a year; (1) from cotton to the winter alternative hosts, (2) from there to the spring alternative hosts and (3) from the latter back again to cotton. Cucurbits and ratoon cotton have been regarded as dangerous sources of infection for the new cotton. Period of quick multiplication of the pest coincides with that of germination and growth of the cotton crop. Severity of attack on cotton extends from June to August. The attack on ratoon cotton continues to be the severest till about the middle of July. Preference for oviposition has been observed for certain host-plants during different periods. Infestation on weeds during the off-season of cotton has indicated that the pest might be checked by practising clean cultivation and removal of weeds.

TABLE I

Comparative infestation of B. gossypiperda *on some of the alternative host-plants during 1932 and 1933*

Host plant	Common name	1932 Average No. of immature stages per sq. inch of leaf area					1933 Average No. of immature stages per sq. inch of leaf area						
		March	April	May	November	December	January	February	March	April	May	November	December
Brassica napus	Indian rape (*Toria*)	...	...	...	3·60	2·50	0·38	0·59	...	...	...	0·67	0·57
Brassica oleracea	Cauliflower	...	...	...	2·28	0·76	0·24	0·77	0·17	...	...	0·45	0·55
Brassica rapa	Turnips	...	...	...	0·32	0·62	0·35	0·37	...	...	...	0·39	0·16
Raphanus sativus	Radish	...	...	...	0·59	0·46	0·34	0·25	0·11	...	...	0·18	0·16
Solanum tuberosum	Potato	...	...	...	37·10	6·10	1·14	1·60	0·48	...	...	0·84	0·66
Lycopersicum esculentum	Tomato	...	...	...	...	...	0·33	0·06	0·46	0·45	...	0·41	0·40
Cucumis melo	Melon	...	2·10	6·60	...	...	...	...	0·62	1·61	2·15	...	...
Cucumis sativus	Cucumber	...	1·04	4·51	...	...	...	...	0·26	1·43	2·72	...	...
Lagenaria vulgaris	Gourd	...	1·11	2·40	...	...	...	...	0·29	0·75	1·32	...	...
Hibiscus esculentus	Lady's finger	...	...	...	...	...	...	...	0·33	1·26	0·78	...	...
Convolvulus arvensis	Bind Weed (*Lehli*)	0·48	0·44	...	2·95	1·05	1·41	1·72	0·10	0·27	...	0·56	1·30
Withania somnifera	*Asgandh* (*Aksin*)	0·52	0·16	...	0·20	0·32	0·40	0·41	0·41	0·21	0·28	0·25	0·16
Achyranthus aspera	*Puthkanda*	0·91	0·78	0·44	0·19	1·31	1·22	0·95	0·39	...	...	0·51	0·36
Sonchus arvensis	Sow thistle	1·50	0·54	...	2·68	2·66	1·50	3·55	6·53	1·15	0·25	1·90	2·60
Euphorbia hypercifolia	*Hazar Dana* (*Dodhak*)	2·70	1·40	2·84	2·70	1·63	...	5·96	...	...	...	0·41	0·87
Solanum nigrum	Black night shade (*Makoh*)	...	...	...	...	...	...	0·57	0·47	0·36	0·23	...	0·42
Ratoon cotton	...	...	...	4·2	...	...	...	...	...	0·38	2·44	...	...

TABLE II

Annual cycle of the distribution of various stages of the White-fly on different host-plants

Duration	*Gossypium* spp. all varieties (New cotton)	*Gossypium* spp. (Ratoon cotton)	*Cucurbit* spp.	*Brassica* spp.	*Nicotiana tabacum*	*Solanum tuberosum*	*Solanum melongena*	*Cyamopsis psoraloides*	*Solanum nigrum*	*Hibiscus esculentus*	*Lycopersicum esculentum*	*Sonchus* spp.	*Withania somnifera*	*Convolvulus arvensis*	*Euphorbia* spp.	*Achyranthus aspera*
1st—15th January . .	E, N.	...	...	E, N.	...	E, N.	...	...	E, N.	...	E, N.	E, N.	E, N.	E, N.	E, N.	E, N.
16th—31st ,, . .	N, P.	...	...	N, P.	...	N, P.	...	...	N, P.	...	N, P.	N, P.	N, P.	N, P.	N, P.	N, P.
1st—15th February . .	...	...	...	A, N, P.	...	A, N, P.	...	...	A, N, P.	...	A, N, P.	A, N, P.	A, N, P	A, N, P.	A, N, P.	A, N, P.
16th—28th ,, . .	...	...	...	A, E, N, P.	...	A, E, N, P.	...	...	A, E, N, P.	...	A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.
1st—15th March . .	...	...	A	A, E, N, P.	A, E, N.	...	A	...	A, E, N. P.	A.	,,	,,	,,	,,	,,	,,
16th—31st ,, . .	...	...	A, E, N.	,,	A, E, N, P.	...	A, E, N.	...	...	A, E, N.	,,	,,	,,	,,	,,	,,
1st—15th April. . .	...	A, E.	A, E, N, P.	...	,,	...	A, E, N, P.	...	...	A, E, N, P.	,,	,,	,,	,,	,,	,,
16th—30th ,, . .	A	A, E, N, P.	,,	...	,,	...	,,	A, E.	...	,,	,,	,,	,,	,,	,,	,,
1st—15th May . . .	A, E, N, P.	,,	,,	...	,,	...	,,	A, E, N, P.	...	,,	...	N, P.	N, P.	N, P.	N, P.	N, P.
16th—31st ,, . .	,,	,,	,,	...	...	...	,,	,,	...	,,	...	...	...	...	...	...
1st—15th June . . .	,,	,,	,,	...	...	...	,,	,,	...	,,	...	...	...	...	...	...
16th—30th ,, . . .	,,	,,	,,	...	...	...	,,	,,	...	,,	...	...	...	...	...	...
1st—15th July . . .	,,	...	...	...	...	...	...	,,	...	...	...	...	...	...	...	...
16th—31st ,, . . .	,,	...	...	...	...	...	...	,,	...	...	...	...	...	...	...	...
1st—15th August . .	,,	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
16th—31st ,, . .	,,	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1st—15th September . .	,,	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
16th—30th ,, . .	,,	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1st—15th October . .	,,	...	...	A, E.	...	A, E.	...	...	...	...	...	...	...	...	...	...
16th—31st ,, . .	,,	...	...	A, E, N.	...	A, E, N.	...	...	A. E.	...	...	A, E.	A. E.	A, E.	A, E.	A, E.
1st—15th November . .	,,	...	...	A, E, N, P.	...	A, E, N, P.	...	...	A, E, N.	...	...	A. E. N.	A, E. N.	A, E,N.	A, E, N.	A, E, N.
16th—30th ,, . .	,,	...	...	A, E, N, P.	...	A, E, N, P.	...	...	A, E, N, P.	...	A, E.	A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.
1st—15th December . .	A, E, N.	...	...	A, E, N.	...	A, E, N.	...	...	A, E, N.	...	A, E, N.	A, E, N.	A, E, N.	A, E, N,	A, E, N.	A, E, N.
16th—31st ,, . .	A, E, N	...	...	A, E, N.	...	A, E, N.	...	...	A, E, N.	...	A, E, N.	A, E, N.	A, E. N.	A, E, N.	A, E, N.	A, E, N.

N. B.—1. A—Adult. E.—Egg. N.—Nymph. P.—Pupa.
2. *Gossypium* spp. include both *desi* and American cottons.
3. *Cucurbit* spp. include *Cucumis melo*; *Cucumis sativus*; *Lagenaria vulgaris*; *Cucumis pubescens*.
4. *Euphorbia* spp. include *Euphorbia prostata*; *Euphorbia pilulifera*; *Euphorbia hyperrifolia*.
5. *Brassica* spp. include *Brassica napus*; *Brassica oleracea*; *Brassica rapa*; *Brassica campestris*.
6. *Sonchus* spp. include *Sonchus arvensis* and *Sonchus oleracea*.

TABLE III

Comparative oviposition of B. gossypiperda, *on cotton and alternative host-plants growing under the same cage*

Host-plants	May			June			July			Host-plants	October			November			December		
	Leaf area sq. in.	No. of eggs laid	Eggs per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.		Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.
Gossypium sp. 4 F.	87·64	379	4·4	149·63	757	5·0	129·34	974	8·9	*Gossypium* sp. 4 F	67·[illegible]	39	0·57	60·70	7	0·11	81·62	9	0·11
Gossypium sp. Mollisoni	76·40	446	5·8	163·02	725	7·3	76·71	847	11·4	*Brassica rapa*	44·[illegible]	42	0·95	71·83	56	0·77	275·06	332	1·20
Althea rosea	57·04	84	1·4	26·12	72	2·7	7·51	16	2·1	*Raphanus sativus*	149·[illegible]	59	0·32	235·18	73	0·31	657·59	655	0·99
Lagenaria vulgaris	153·37	645	4·2	166·49	669	4·0	114·13	347	3·0	*Brassica oleracea*	52·31	113	2·16	142·15	283	1·99	369·48	663	1·79
Luffa acutangula	165·43	468	2·8	153·69	498	3·7	73·04	208	2·8	*Solanum melangena*	35·33	16	0·45	23·99	19	0·8	14·62	17	1·16
Cucumis sativus	101·93	550	5·3	155·55	498	3·1	123·64	487	3·9	*Brassica napus*	...	...	...	...	...	...	54·24	178	3·28
Cucumis melo.	141·72	1,017	7·1	110·79	797	7·8	107·59	473	4·3	*Solanum tuberosum*	...	...	...	...	...	...	41·85	106	2·53
										Lycopersicum esculentum	...	...	...	...	...	...	62·22	217	3·48

TABLE IV

Showing the above figures arranged in order of preference regarding the available leaf area and the density of population

Host-plants	May		June		July		Host-plants	October		November		December	
	In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs		In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs
Gossypium sp. 4 F.	V	(6)	III	(2)	II	(1)	*Gossypium* sp. 4 F	III	(4)	IV	(5)	IV	(8)
Gossypium sp. Mollisoni	VI	(5)	V	(3)	V	(2)	*Brassica rapa*	IV	(3)	III	(3)	III	(3)
Althea rosea	VII	(7)	VII	(6)	VII	(7)	*Raphanus sativus*	II	(2)	I	(2)	I	(2)
Lagenaria vulgaris	II	(2)	I	(4)	III	(5)	*Brassica oleracea*	III	(1)	II	(1)	II	(1)
Luffa acutangula	I	(4)	IV	(5)	VI	(6)	*Solanum melongena*	V	(5)	V	(4)	VIII	(7)
Cucumis sativus	IV	(3)	II	(5)	I	(3)	*Brassica napus*	...	...	...	...	VI	(5)
Cucumis melo.	III	(1)	VI	(1)	IV	(4)	*Solanum tuberosum*	...	...	...	...	VII	(6)
							Lycopersicum esculentum	...	...	...	...	V	(4)

TABLE V

Relative infestation of B. gossypiperda, *on Mollisoni and 4-F varieties of cotton*

Variety	Year	Average number of immature stages per square inch of leaf area													
		May Fortnight		June Fortnight		July Fortnight		August Fortnight		September Fortnight		October Fortnight		November Fortnight	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II
Mollisoni	1931	...	...	12·3	14·1	23·0	30·9	27·8	12·4	3·2	1·5	1·3	0·6	0·4	0·3
4-F.		...	...	3·3	6·5	13·6	18·7	19·7	14·5	4·3	2·4	2·0	0·9	0·7	0·2
Mollisoni	1932	...	10·8	33·5	45·5	49·3	55·1	40·4	11·7	1·6	1·1	0·7	0·3	0·5	0·5
4-F.		6·1	6·1	7·1	16·1	22·2	38·4	34·3	14·0	3·0	1·0	0·6	0·3	0·2	0·3
Mollisoni	1933	...	...	12·8	11·4	2·9	1·7	0·5	0·4	0·3	0·4	0·1	0·1	0·3	0·8
4-F.		...	...	6·1	2·2	2·2	1·3	0·5	0·1	0·2	0·2	0·1	0·2	0·1	0·1

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STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

II. SOME STUDIES IN THE PHYSIOLOGY OF THE CAUSAL FUNGI

BY

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(With two text-figures)

CONTENTS

	PAGE
I. FUNGI STUDIED	905
II. MEDIA EMPLOYED	905
III. CULTURAL CHARACTERS OF THE TWO *Rhizoctonia* SPP.	907
IV. GROWTH BEHAVIOUR OF THE PATHOGENE UNDER DIFFERENT CONDITIONS OF NUTRITION	909
V. EFFECT OF DIFFERENT pH CONCENTRATIONS ON GROWTH	910
VI. EFFECT OF CARBON DIOXIDE	912
VII. EFFECT OF NITROGEN	913
VIII. THERMAL DEATH POINT	913
IX. EFFECT OF MIXTURE OF ORGANISMS ON THE ACTIVITY OF THE TWO *Rhizoctonia* spp.	913
X. SUMMARY	916
REFERENCES	916

PREFACE

An investigation of the root-rot disease of cotton in the Punjab was taken in hand by Dr. R. S. Vasudeva, Assistant Cotton Mycologist, appointed for the purpose in the Scheme financed by the Indian Central Cotton Committee since 1932.

During the first three years of the Scheme, most of the time has been devoted to the discovery of causal organisms of the disease. The results definitely establish that the disease is caused mainly by two fungal parasites, namely *Rhizoctonia bataticola* and *Rhizoctonia solani*. A paper giving an account of the experimental work carried out in this connection leading to definite conclusions with regard to the pathogenic nature of the disease was published in this *Journal* (1935). In the present paper, which is in continuation of the previous one, is described how the various fungi react with regard to parasitism and growth on different media alone and in combination with some non-parasitic fungi that were isolated from the roots of cotton plants affected by the disease. The know-

ledge gained on the pathology of the causal organisms of the disease is likely to indicate the way in which the parasites bring about injury to the roots of the host-plants.

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I. FUNGI STUDIED

The investigation reported in the paper was carried out with the following fungi isolated from the roots of cotton plants affected by the root-rot disease :—

1. A fungus provisionally *called *Rhizoctonia bataticola.*
2. *Rhizoctonia solani.*
3. Strain A of *Fusarium solani.*
4. Strain B of *Fusarium solani.*
5. A species of *Alternaria.*
6. A species of *Helminthosporium.*
7. *Fusarium species* (15).
8. *Fusarium species* (17).

Before commencing the work, the purity of the fungi was ensured by taking a single hyphal tip in each case, after the method described by Brown [1924] and stock cultures were maintained in tubes of potato extract agar.

II. MEDIA EMPLOYED

The following media with or without agar were used :—

1. Glucose peptone—

Glucose	10 grms.
Peptone	2 „
Potassium dihydrogen-phosphate	1 grm.
Magnesium sulphate	0·5 „
Distilled water	1,000 c. c

2. Brown's synthetic solution.
3. Potato extract—

Peeled potatoes	20 grms.
Distilled water to make up	1,000 c. c.

4. †Cotton root extract—

Cotton roots	200 grms.
Distilled water to make up	1,000 c. c.

*The systematic position of this fungus has not yet been exactly determined. It will be given in a later publication.

† Cotton roots or leaves were cut into small pieces, covered with distilled water and autoclaved at 15 lbs. pressure for one hour and passed through muslin. The extract thus obtained was made up to 1,000 c.c. by adding distilled water.

5 Cotton leaf extract—

Cotton leaves	200 grms.
Distilled water to make up	1,000 c. c.

6. Richards' agar.

7. Cotton root synthetic.

As the cotton root extract, though quite suitable for culture of the two *Rhizoctoniaspecies*, is liable to vary in composition ; it was thought that if a synthetic substitute could be found it would prove of great advantage and the results would be strictly comparable. In proceeding to evolve such a medium, a start was made by analysing chemically, roots of *desi* cotton (Mollisoni 15). The roots belonged to the plants which were about five months old. The average composition of the roots thus analysed is given below :—

Moisture, 65·00 per cent.		Fat	0·37 per cent.
		Protein	1·45 per cent.
Dry matter, 35·00 per cent.		N-free extract	10·49 per cent.
		Fibre	21·05 per cent.
		Ash	1·64 per cent.

As regards the constituent of ash, 1·64 grms. of it contain :—

Fe_2O_3	0·02 grm.
AI_2O_3	0·02 „
Ca O	0·25 „
Mg O	0·06 „
Mn_3O_4	0·01 „
Na_2O	0·02 ,
K_2O	0·63 „
P_2O_5	0·08 „
S O_4	0·16 „
Insoluble residue	0·22 „

A decoction of 200 grms. of cotton roots in the litre of water contains the following :—

2·90 grms. nitrogenous substance.
0·74 grm. fat.
20·98 grms. nitrogen-free extract.
42·10 grms. fibre.
3·28 grms. ash.

In preparing the solution, the fat and fibre constituents of the organic part were ignored, as were also the sodium and aluminium constituents of the ash. The latter two were omitted as there is a strong evidence that these elements in general are not essential for fungal growth.

The remaining constituents of the cotton root extract were then added in the following form and proportion. The proportion was calculated to give as closely

as possible the constituents and quantities required according to the analytical data given above.

Glucose	20 grms.
Peptone	2·9 „
$K_3 PO_4$	1·9 „
*$Ca_3 (PO_4)_2$	0·9 „
$MgSO_4$	0·4 „
NaCl	0·6 „
$FeCl_3$	A trace.
Distilled water to make up	1,000 c.c.

This medium has proved more suitable for the growth of the two *Rhizoctonia* spp. than the other artificial media employed, as the growth on this synthetic medium is comparable and almost identical with that obtained on the decoction of the natural host.

III. Cultural characters of the two *Rhizoctonia* spp.

The fungi were grown on Brown's agar, Richards' agar, cotton leaf and root extracts, and cotton root synthetic at a temperature of 20°C., 25°C., 30°C., 35°C., 40°C. and 45°C., respectively. The plates used were of uniform diameter and into each was poured 50 c.c. of the medium in order to maintain uniformity in depth. These experiments were run in quadruplicate.

* $Ca_3 (Po_4)_2$ is very slightly soluble in water and may be omitted without any appreciable difference in growth.

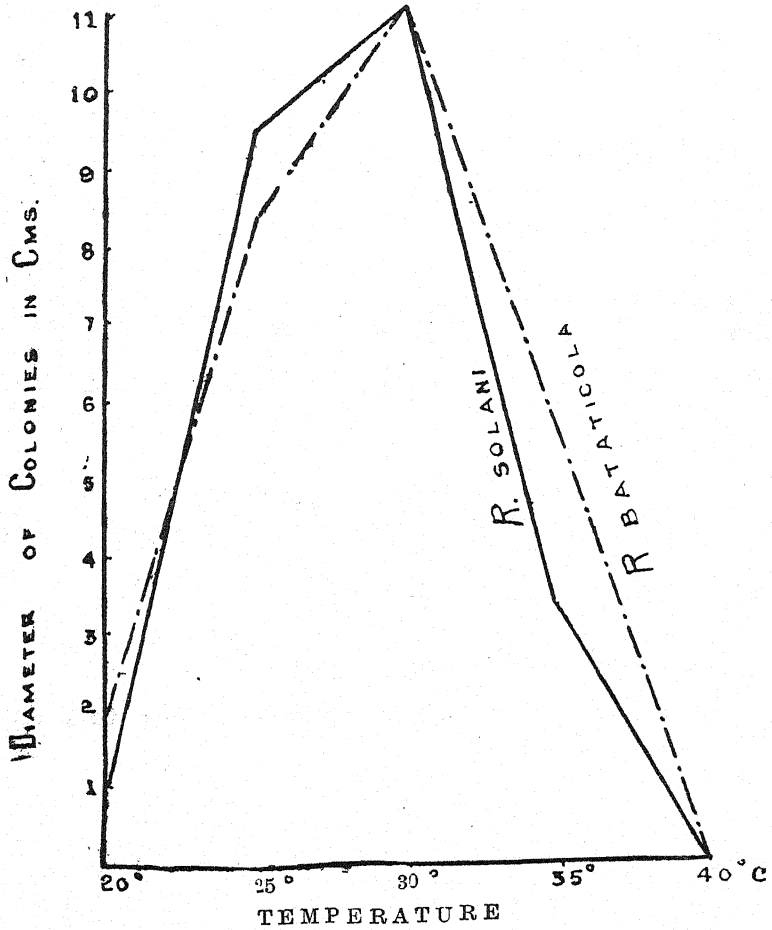

Fig. 1. Showing effect of temperature on rate of growth of *R. bataticola* and *R. solani* on cotton root synthetic agar (3 days' growth).

The relation between temperature and linear growth three days after inoculation is shown in Fig. 1 which gives the rate of spread of *Rhizoctonia bataticola* and *Rhizoctonia solani* on cotton root synthetic over a series of temperatures.

Both are fairly fast growing fungi with optimum growth at 30°C. Above 30°C. there is a fall in the rate of growth and a cessation occurs at 40°C. on all the media with the exception of Brown's agar on which *Rhizoctonia bataticola* exhibited slight growth even at 40°C. But no growth occurred at 45°C. even on this medium.

Rhizocotonia solani produces plenty of profuse white aerial mycelium which turns light brown with age. There are plenty of white aggregations of hyphae which later form dark brown hard sclerotia.

Rhizoctonia bataticola produces a white colony with plenty of aerial mycelium which turns dark grey with age and has numerous tiny dark sclerotial bodies. It does not produce pycnidia or spores of any kind.

IV. Growth behaviour of the pathogene under different conditions of nutrition

The growth behaviour of the two *Rhizoctonia* spp. was studied on cotton root synthetic solutions. The importance of each constituent of the synthetic solution was determined by preparing solutions lacking in that constituent. The two fungi were grown in 100 c.c. Erlenmeyer flasks containing 30 c.c. of the medium. The experiment was carried out in triplicate.

The flasks were inoculated after sterilization in the usual way with cultures of the fungi growing on potato-extract agar. Great care was taken and an inoculum of almost uniform size was introduced into each flask. The hydrogen-ion concentrations of uninoculated media were determined by Clark's method soon after sterilization. The dry weight of the fungal mats and the pH values of the stales were determined after the fungi had grown in cultures for twenty-four days. The fungal material was collected on filter papers by means of a Buchner filter, dried in an oven, cooled in a calcium chloride desiccator, and weighed.

The results of the experiment are given in Table I.

Table I

Weights of the mycelium of Rhizoctonia bataticola *and* Rhizoctonia solani *and pH of filtrates after twenty-four days' growth in various modifications of cotton root synthetic*

Medium	*Rhizoctonia bataticola*			*Rhizoctonia solani*		
	Average dry weight of mycelium in mg.	pH of un-inoculated control solution	pH of filtrates	Average dry weight of mycelium in mg.	pH of un-inoculated control solution	pH of filtrates
Complete cotton root synthetic	109	5·6	6·4	133	6·2	7·2
Complete medium—glucose .	20	8·0	8·0	11	7·8	8·2
Complete medium—peptone .	7	5·4	5·8	9	6·0	6·4
Complete medium—K_3PO_4 .	80	5·2	7·4	82	6·0	8·0
Complete medium—$Ca_3(PO_4)_2$	107	5·4	7·0	151	6·0	6·8
Complete medium—$MgSO_4$.	130	5·4	6·8	93	5·8	7·4
Complete medium—NaCl .	173	5·4	6·6	133	6·0	6·8
Complete medium—$FeCl_3$.	200	5·4	6·8	173	6·2	6·8
Complete medium—both K_3PO_4 and $Ca_3(PO_4)_2$.	63	4·8	5·0	83	5·4	6·0

(— lacking in).

As is clear from the above table, the omission of magnesium and sodium salts has little effect on the amount or kind of growth, whereas in the absence of iron salts the growth is comparatively better and more luxuriant. When sugar is omitted, the vigour of growth is considerably reduced. In the absence of peptone, the growth is reduced to a minimum and is in the form of a thin scanty film. Next in importance is the phosphate constituent in the absence of which the vigour of growth is also reduced.

Both the *Rhizoctonia* spp. tend to change the reaction of the medium and in all cases reduce the acidity of the solutions.

V. Effect of different pH concentrations on growth

For the investigation of influence on growth of media varying in pH value from 2·4 to 9·2, the two *Rhizoctonia* spp. were grown on glucose-peptone agar prepared with different degrees of acidity and alkalinity by addition of malic acid and sodium bicarbonate respectively. This medium is normally acid, 100 c.c. requiring approximately 0·07 grm. of sodium bicarbonate for neutralisation. Fifteen modifications of the medium were made up showing a range of acidity and alkalinity. The Petri dishes were inoculated in triplicates and the linear growth rates measured from day to day.

Fig. 2 shows diameters of colonies of *Rhizoctonia bataticola* and *Rhizoctonia solani* on media of different pH values.

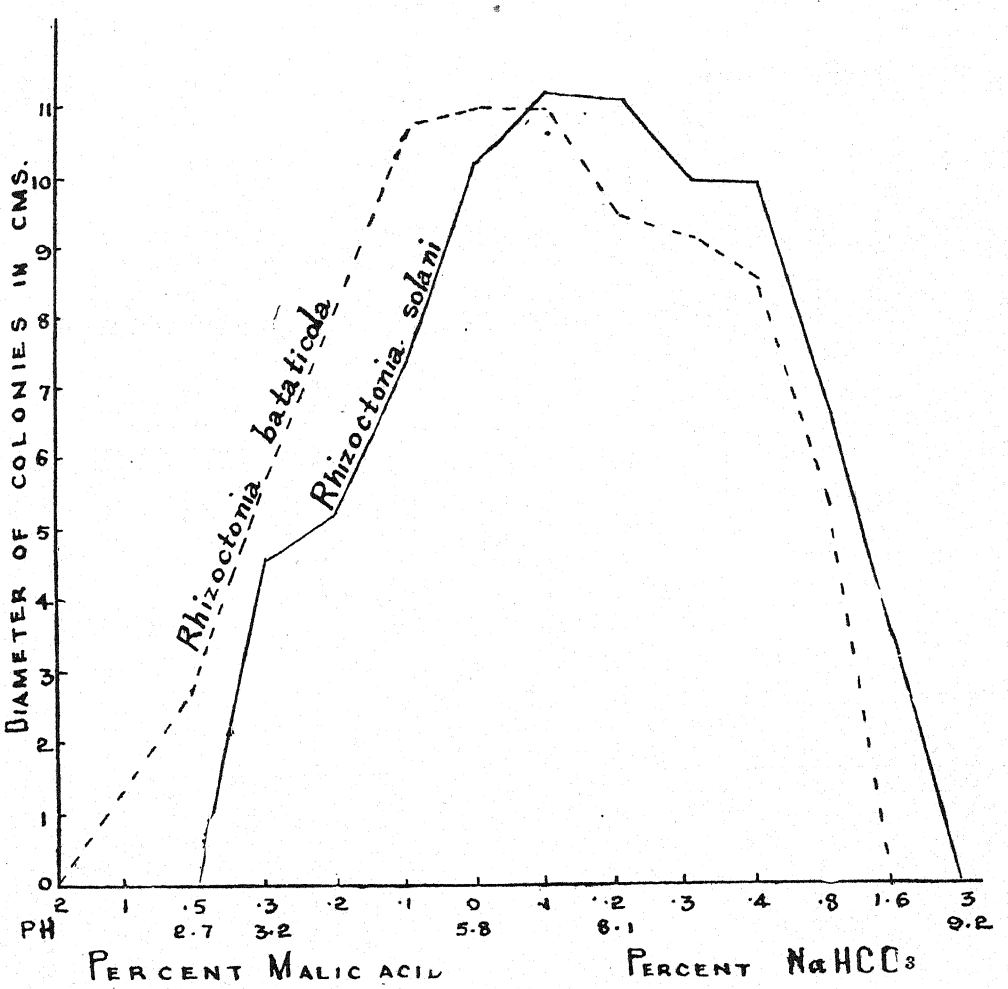

Fig. 2. Showing diameters of colonies (three days' growth) of *Rhizoctonia bataticola* and *Rhizoctonia solani* on media of different pH (Medium: Glucose-peptone).

It is evident from the data obtained that both the fungi have a very wide range of toleration to acidity and alkalinity, that is between pH 2·4 and 9·2 with fairly good growth between pH 3·2 and 8·5 and optimum near the neutral point. This experiment was repeated with cotton root synthetic as basal medium and similar results were obtained.

VI. Effect of carbon dioxide

The two *Rhizoctonia* spp. were grown in different concentrations of carbon dioxide, *e.g.*, twenty-five, fifty, seventy-five and hundred per cent on Richards' agar in quadruplicates. Controls were kept under ordinary atmospheric conditions.

The inoculated plates were kept in desiccators which were then sealed.

The carbon dioxide was let into the sealed chambers by creating the required amount of vacuum by adjustment of pressure by a manometer.

The results obtained by growing the fungi in normal atmosphere and in varying concentrations of carbon dioxide are given in Table II.

Table II

Effect of carbon dioxide on the growth of Rhizoctonia bataticola *and* Rhizoctonia solani

Fungus	Concentration of CO_2 per cent	Diameter of the colonies in centimeters (after 5 days' growth) Plate numbers				
		1	2	3	4	Average
Rhizoctonia bataticola	25	4·8	5·2	5·5	5·3	5·20
Rhizoctonia solani		1·5	1·2	1·6	1·3	1·86
Rhizoctonia bataticola	50	2·8	2·7	2·1	2·4	2·50
Rhizoctonia solani		0·0	0·0	0·0	0·0	0·00
Rhizoctonia bataticola	75	0·6	0·5	0·6	0·5	0·55
Rhizoctonia solani		0·0	0·0	0·0	0·0	0·00
Rhizoctonia bataticola	100	0·3	0·2	0·2	0·2	0·22
Rhizoctonia solani		0·0	0·0	0·0	0·0	0·00
Rhizoctonia bataticola	Control	10·4	10·2	10·4	10·6	10·40
Rhizoctonia solani		7·5	7·1	7·4	7·2	7·27

At fifty per cent concentration of carbon dioxide the growth of *Rhizoctonia solani* altogether ceased whereas a slight growth took place in the case of *Rhizoctonia bataticola* even at 100 per cent carbon dioxide concentration. At any rate marked reduction in growth was noticed in both the fungi even at twenty-five per cent carbon dioxide concentration. Though the growth is arrested at higher concentrations of carbon dioxide, yet the fungi are found to be viable even after forty-four days' exposure to pure carbon dioxide when normal atmospheric conditions are restored.

In another similar experiment twenty-five per cent and fifty per cent vacuum were created in sealed chambers but no carbon dioxide was introduced.

Here even at fifty per cent vacuum the difference in the amount of growth when compared with the controls was slight. This clearly shows that the depressing effect shown above, is only due to the introduction of carbon dioxide and not due to deficiency of ordinary atmosphere (oxygen).

VII. Effect of nitrogen

The effect of the atmosphere of nitrogen on the growth of *Rhizoctonia bataticola* and *Rhizoctonia solani* was studied by placing the inoculated Petri dishes in desiccators in which normal atmosphere had been replaced by nitrogen by the pyrogallic acid method. The difference between the rates of growth of the control and of those exposed to the atmosphere of nitrogen was very slight. But there was a marked difference in the amount of aerial mycelium. In the plates exposed to the atmosphere of nitrogen, the growth was thin, scanty and spreading with little aerial mycelium.

VIII. Thermal death point

In order to determine the thermal death point of the two *Rhizoctonia* spp. a suspension of the fungal mycelium was prepared in sterile water. Some narrow glass tubing which had been drawn into capillary form was cut into pieces two inches in length. Then each of these tubes was fused in the middle, thus making one inch capillary tubes sealed at one end. These tubes were then dropped into the water suspension of the fungal mycelium and exhausted under air pump. By this means, the capillary tubes got filled with the suspension. They were then placed in beakers of water at various temperatures ranging from 45 to 70° C. for five seconds ; capillary tubes so treated were then broken off at the ends and the contents collected on Richards' agar in Petri dishes. It was observed that *Rhizoctonia solani* failed to grow when exposed at 60° C. for five seconds but in the case of *Rhizoctonia bataticola* an exposure of five seconds at 68° C. was required to kill the fungus. A similar experiment to determine the thermal death point of the sclerotia of the two *Rhizoctonia* spp. was carried out but this revealed no difference between the thermal death point of the mycelium and the resting bodies.

Capillary tubes filled with a suspension of fungal mycelium of *Rhizoctonia bataticola* and *Rhizoctonia solani* were exposed to 0°C. The viability tests were carried out after exposure from 1 to 120 hours but the fungi were found to be viable.

IX. Effect of mixture of organisms on the activity of the two *Rhizoctonia* spp.

In a preceding paper of this series [Vasudeva ; 1935, 1] it was shown that the two *Rhizoctonia* spp. almost always appeared together when isolations were made

from the diseased roots of cotton and were the cause of root-rot disease of cotton in the Punjab. In addition several strains of *Fusarium*, a species of *Alternaria* and *Helminthosporium* were isolated.

The effect of the parasitic activity of the two *Rhizoctonia* spp. when occurring singly or in mixtures either with each other or with one of the other organisms mentioned was a matter which attracted considerable attention. The two strains of *Fusarium* which were later identified as those of *Fusarium solani*, a species of *Alternaria* and *Helminthosporium* and in addition two more strains of *Fusarium* did not produce the symptoms of root-rot disease.

Experiments were carried out with batches of cotton plants of 15 Mollisoni and 4 F varieties respectively.

The general plan of the experiment was to inoculate batches of cotton plants as follows :—

(*i*) A batch of cotton plants with parasitic fungus alone.

(*ii*) A batch of cotton plants with non-parasitic fungus.

(*iii*) A batch of cotton plants with active+inactive fungus.

(*iv*) A batch of cotton plants as controls.

The inoculations were made in a standardized way by exposing the roots and adding a uniform amount of inoculum by weight and covering up by means of the sterilized soil. In the case of the controls the fungal inoculum was not introduced.

In all the four batches uniform conditions were maintained throughout the experiment whether carried out in laboratory or in field.

In some experiments both the active fungi, *i.e.*, the two *Rhizoctonia* spp. were introduced together and in others either of them singly was used.

The results of a typical experiment in which the active organism used was *Rhizoctonia solani* are set out in Table III.

TABLE III

Variation in the parasitic activity of Rhizoctonia solani *in the presence of other organisms*

Inoculum	No. inoculated	No. wilted	Percentage of mortality
Rhizoctonia solani	10	2	20·00
Rhizoctonia solani+*Fusarium solani* (*A*) . .	6	2	33·33
Rhizoctonia solani+*Fusarium* sp. (15) . . .	8	8	100·00
Rhizoctonia solani+*Fusarium* sp. (17) . . .	4	4	100·00
Rhizoctonia solani+*Fusarium sol.* (*B*) . . .	6	2	33·33
Rhizoctonia solani+*Helminthosporium* sp. . .	6	3	50·00

Duration of the experiment was one week.

These are the results of an experiment carried out under controlled laboratory conditions. Controls in all cases remained unaffected. All the comparisons shown above indicate that the parasitic activity of *Rhizoctonia solani* is enhanced when it acts in combination with other fungi.

On repeating the experiment similar results were obtained. These results are in contrast with those obtained by Vasudeva [1930] for *Monilia fructigena* and *Botrytis cinerea* on apple, where the activity of the organisms was considerably reduced in mixed inoculations. But these are in accordance with Fawcett [1923] who obtained increased parasitic activity of *Pythiacystis citrophthora* in presence of a species of a *Fusarium* in the inoculum and with Vasudeva [1935, 2] who observed increased parasitic activity of *Rhizoctonia solani* in the presence of species *Alternaria* and *Helminthosporium* on turnips.

When *Rhizoctonia bataticola* is introduced into the inoculum in combination with the other fungi, *i.e.*, various strains of *Fusarium* species of *Helminthosporium* and *Alternaria*, there is no significant difference in the parasitic activity.

The same type of experiment was repeated using either of the *Rhizoctonia* spp. singly against both of them acting together. The results from six such experiments carried out in the laboratory are given in Table IV.

TABLE IV

The parasitic activity of the two Rhizoctonia *spp. when parasitising singly and jointly under controlled laboratory conditions*

Experiment	Inoculum	No. inoculated	No. wilted	Percentage mortality	Controls
I	*Rhizoctonia solani* . . .	15	3	20·00	13, all sound
	Rhizoctonia bataticola . .	15	5	33·33	
	R. solani+*R. bataticola* . .	12	12	100·00	
II	*Rhizoctonia solani* . . .	11	1	9·09	9, all sound
	Rhizoctonia bataticola . .	12	11	91·67	
	R. solani+*R. bataticola* . .	9	9	100·00	
III	*Rhizoctonia solani* . . .	13	3	23·08	11, all sound
	Rhizoctonia bataticola . .	11	9	81·82	
	R. solani+*R. bataticola* . .	11	9	81·82	
IV	*Rhizoctonia solani* . . .	11	2	18·18	11, all sound
	Rhizoctonia bataticola . .	10	1	10·00	
	R. solani+*R. bataticola* . .	12	8	66·67	
V	*Rhizoctonia solani* . . .	12	1	8·33	12, all sound
	Rhizoctonia bataticola . .	13	12	92·31	
	R. solani+*R. bataticola* . .	12	12	100·00	
VI	*Rhizoctonia solani* . . .	5	2	40·00	5 all sound
	Rhizoctonia bataticola . .	4	..	..	
	R. solani+*R. bataticola* . .	4	4	100·00	

The results shown above indicate that the parasitic activity of *Rhizoctonia solani* tends to increase when acting in combination with *Rhizoctonia bataticola*.

Increased parasitic activity is also shown by *Rhizoctonia bataticola* when parasitising in combination with *Rhizoctonia solani*. In the 3rd experiment however, its parasitic activity remains unchanged.

In conclusion I wish to place on record my thanks to Professor Rai Sahib Jai Chand Luthra for the keen interest throughout the course of this investigation. Thanks are also due to the Agricultural Chemist for the help rendered in carrying out the chemical analysis of the cotton roots.

X. Summary

1. A synthetic nutrient medium equivalent to the composition of cotton roots has been described.

2. The dependence of growth upon the most important constituents of the nutrient medium has been studied.

3. Both the *Rhizoctonia* spp. are fairly fast-growing fungi with optimum growth at 30°C. and have a very wide range of toleration to acidity and alkalinity, that is between pH 2·4 and 9·2.

4. Carbon dioxide has a depressing effect on the growth of *Rhizoctonia bataticola* and *Rhizoctonia solani*.

5. Upper thermal death points of *Rhizoctonia bataticola* and *Rhizoctonia solani* are very high, being 68°C. and 60°C. respectively.

6. The parasitic activity of *Rhizoctonia solani* is increased in the presence of certain other fungi in the inoculum.

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ON SOME OF THE FACTORS THAT INFLUENCE THE ERROR OF FIELD EXPERIMENTS WITH SPECIAL REFERENCE TO SUGARCANE

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INTRODUCTION

The successful interpretation of field experiments of modern days depends to a large extent on the correct estimation of the error due to chance. But agricultural experiments are subject to errors due to a multiplicity of causes, and most of them are beyond control. The heterogeneity of the soil, the conditions of temperature, humidity and rainfall, etc., that vary from year to year are factors beyond man's control. The crops on the border have more sunshine, more air and more space for growth than those in the interior which have to struggle among themselves for a requisite supply of these natural factors for their full development and growth. The natural result is that the border crops are usually better than the interior and this high yield of the border region increases the mean and the variance. In manurial experiments there is always a certain amount of admixture of treatments between adjoining plots by leaching. Analyses of results of experiments in Europe and America, specially designed, have proved conclusively that size and shape of plots exercise a good deal of influence on the error of an experiment.

Considering the various factors that come into play in the conduct of agricultural experiments in the field, one cannot help concluding that field experiments can never be performed under ideal conditions. Under the circumstances, the best thing that can be done is to eliminate as far as possible the error that may be introduced due to known causes. In designing the plan of a field experiment, particular attention may be paid to statistical requirements so as to reduce the errors to a practical minimum.

Another important factor which influences any estimate of error is the number of replications. The accuracy of the variance and the mean depends on the number of replications used. It has been found that the precision rapidly increases as the replications are increased. But after a certain stage, the increase

in the accuracy is not commensurate with the labour and money spent on the increase in replications. Hence the minimum number of replications required to satisfy the demands of statistics may be determined.

Not much work has been done in India as to the best size of plots suited for experiments on sugarcane. In this paper it is proposed to determine a suitable size of plots and the number of replications required so that the variance may be reduced to a practical minimum for experiments on cane.

Review of literature

It would be difficult indeed to review within the compass of a short paper like this, the vast amount of literature that has steadily accumulated during the last two decades on the size of plots for agricultural experiments, not necessarily sugarcane. We are content to make only a passing reference to some of the more important contributions made by agricultural workers in Europe, America and India on the subject and the broad conclusions arrived at by them.

Mercer and Hall [1911] were the pioneer workers in the field, and they find that in field trials, however uniform and careful the management may be, there will be an experimental error attached to the result due to so many incalculable factors that may be described as casual. The error diminishes with the size of the plot, but the reduction is small when the area of the plot is above 1/40 acre. The error can be diminished by increasing the number of replications, but there is not much to be gained by increasing the number above five. Day [1920] concludes his paper on the relation of size, shape and number of plots to probable error to field experimentation with the remark that increasing the size of plot to 1/20 acre and probably much beyond that reduces variation. More accurate results are obtained by long and narrow plots extending in the direction of greatest variation. An increase in the number of replications increases the accuracy of the result. Variation is reduced when the size of plots is increased keeping the number of replications constant. Wood and Stratton [1910] remark that the probable error does not depend on the size of the plot. It is not larger for smaller plots of 1/40 or 1/80 acre. In large plots, the errors due to individual variation and inaccuracies of weighing are reduced. But this reduction is balanced by systematic errors due to variation of soil which is greater for bigger plots. Christidis [1931] in a general discussion on the effect of shape of plots in the reduction of error in field experiments finds that a square plot is no way superior to long and narrow ones. According to him there are some practical considerations which may make it desirable to increase the width of the plot in relation to its length. Thompson [1934] in his study on the influence of plot size, shape, orientation and the number of replications on the experimental error on sweet-potatoes draws the following conclusions :—Variations among plots increase as the plot approaches a square. It is not advisable to suggest that any one plot size would be the most desirable.

Equally satisfactory results may be obtained from plots of different sizes provided they are replicated a sufficient number of times. Immer [1932] in his investigations on size and shape of plots in relation to field experiments with sugar beet notices that standard error decreases in general with increased size of plot. Efficiency in use of land also decreases as the size of the plot increases. Sayer and Vaidyanathan [1936] recently found with reference to unirrigated sugarcane that an experimental plot-size between 12/242 acre and 16/242 acre is desirable, that the ratio of length to width of the experimental plot should be at least 6·7 and that the minimum number of replications required is four.

Material

In continuation of the previous years' experiments with Co. 205 and Co. 213, the results of which will be published shortly, under the joint authorship of Wynne Sayer and Rao Bahadur M. Vaidyanathan, another experiment was laid out with Co. 210 during the year 1933-34. The choice for this particular variety was made for the reason that Co. 210 like Co. 213 is recognised as the standard variety of cane of North Bihar and grown under unirrigated condition. All cane experiments, manurial and cultural, are laid down mostly under this variety or Co. 213.

The present experiment was laid out at the New Area, Pusa 'A' Block, on an area of six acres. This land is solely devoted to sugarcane experiments and forms a separate unit for this purpose. The land is of light nature and represents the average soil of the district on which cane is grown for the sugar industry. It is farmed on a regular system of crop rotation that has been found suitable for cane growing on the type of soil represented in this area. The previous cropping history and the rotation are given below :—

Year	*Kharif*	*Rabi*
1929-30	Cane	Cane
1930-31	Pulses	Oats
1931-32	Maize	Berseem
1932-33	Pulses	Fallow
1933-34	Cane	Cane

The experiment was laid out in six blocks, each separated by a three-feet bund. The cane was planted eye to eye in rows three feet apart with six feet of the ends doubled on the 20th February, 1933. The standard manure (40 lb. nitrogen as rape-cake and 50 lb. P_2O_5 as superphosphate) which forms the usual dressing for all cane crop on the Farm was applied at the time of planting. The

operation of earthing up the rows was completed just before the break of monsoon. The record of rainfall during the year of the experiment is given below :—

February, 1933	0·26	inches
March „	*Nil*	
April „	1.38	„
May „	4·09	„
June „	6·75	„
July „	21·69	„
August „	11·35	„
September „	5·45	„
October „	1·89	„
November „	*Nil*	
December „	*Nil*	
January 1934	0·59	„
Total	53·43	inches

Average rainfall for twenty years=47·00 inches.

At harvest, the crop was fairly uniform in growth and there was very little lodging.

The cutting of canes was started on the 3rd January, 1934 and it took twenty-four days to finish the entire work. It would have been possible to complete the work seven days earlier, but the earthquake of the 15th January caused interruption and the work was consequently prolonged.

Before harvesting for final yields, the border crop with a minimum width of six feet was removed from all sides of the six blocks. As a result of this it will be seen in the Appendix, an alley was formed between the adjacent blocks. Each block measured 240 feet in length and was divided into four equal sections. The ultimate plot thus obtained consisted of one row a piece and measured 60 ft. × 3 ft. or 1/242 acre. The canes from these plots were cut, stripped and weighed separately guarding against all possible sources of error. The area thus harvested after excluding border crop was four acres and consisted of 121 rows of 480 ft. in length excluding the alley between the adjacent plots. The number of yield figures recorded was 968.

Methods employed for the statistical analysis

Plot size.—Tables giving the yields for various plot sizes, *viz.*, 60 ft.×3 ft., 120 ft.×3 ft., 60 ft.×6 ft.....up to 24/242 acre, were drawn up and the coefficient of variation after eliminating the soil variability was calculated for the respective plot sizes. In getting the yield for different areas, care was taken to see that the figures from one block were not combined with any of the other blocks. In adopting such a procedure, a few observations from each block for certain combinations had to be omitted.

The standard error was calculated after eliminating the error due to rows and columns. The method adopted will be clear from the tables of analysis of variance given below for plot sizes 60 ft. × 3 ft. and 240 ft. × 9 ft.

TABLE I

Analysis of variance (60 ft. × 3 ft.)

Variance due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Rows . . .	120	566111·7356	..	..
Columns . . .	7	210908·2843	..	..
Residual . .	840	954366·4344	1136·1505	33·71
Total .	967	1731386·4543	1790·4720	42·31

TABLE II

Analysis of variance (240 ft. × 9 ft.)

Variance due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Rows .	38	5592808·3490	..	..
Columns .	1	1458097·1599	..	..
Residual .	38	4263415·2151	112195·1372	334·96
Total .	77	11314320·7240	146939·2300	383·33

Table III gives for each combination the size of plots, the number of rows and columns, the mean, the standard deviation and the coefficient of variation after eliminating soil variability. In columns 10 and 11 are given the standard deviation and the coefficient of variation without any elimination. This will enable us to judge the extent to which the error due to soil heterogeneity has been eliminated. The approximate number of replications necessary, so that the difference between the mean of the sample and the population to which the sample belongs is five per cent or ten per cent of the population mean, is also given in the last two columns of the table for various plot sizes. The methods adopted for determining these have been indicated briefly in the course of the discussion of the results.

TABLE III

Results of analysis of data for plot size and shape

Serial No.	Plot size	Area (acre)	Ratio of length to width	No. of rows	No. of columns	Mean	After elimination of soil variation in two directions		Without any elimination		Number of replications Odds 1 : 30	
							S. D.	C. V.	S. D.	C. V.	5 per cent	10 per cent
1	2	3	4	5	6	7	8	9	10	11	12	13
1	60 ft. × 3 ft.	1/242	20	121	8	270·8290±1·0835	33·71±0·7662	12·4470±0·2873	42·31	15·6224	25	9
2	60 ft. × 6 ft.	2/242	10	60	8	541·4021±2·8162	61·70±1·9914	11·3964±0·3726	79·37	14·6601	22	8
	120 ft. × 3 ft.	2/242	40	121	4	541·6581±2·6832	59·03±1·8973	10·8980±0·3544	75·05	13·8556	21	7
3	60 ft. × 9 ft.	3/242	6·7	39	8	810·0144±4·9769	87·91±3·5192	10·8529±0·4754	114·45	14·1294	21	7
	180 ft. × 3 ft.	3/242	60	121	2	813·5165±5·1130	79·54±3·6154	9·7773±0·4487	102·60	12·6119	17	7
4	60 ft. × 12 ft.	4/242	5	29	8	1083·5388±7·7182	117·56±5·4576	10·8496±0·551	153·60	14·1758	21	7
	120 ft. × 6 ft.	4/242	20	60	4	1082·3042±7·1631	110·97±5·0651	10·2484±0·5136	143·12	13·2175	18	7
	240 ft. × 3 ft.	4/242	80	121	2	1083·3161±7·3571	114·45±5·2023	10·5649±0·5263	134·00	12·3695	20	7
5	60 ft. × 15 ft.	5/242	4	22	8	1347·5398±10·8996	144·60±7·7072	10·7307±0·6263	184·88	13·7198	20	7
6	60 ft. × 18 ft.	6/242	3·3	19	8	1624·0987±13·9291	171·73±9·8493	10·5739±0·6646	222·46	13·6974	20	7
	120 ft. × 9 ft.	6/242	13·3	39	4	1620·0288±13·1713	164·51±9·3135	10·1548±0·6315	210·57	12·9979	18	7
	180 ft. × 6 ft.	6/242	30	60	2	1626·4333±13·9012	152·28±9·8296	9·3628±0·6097	197·16	12·1222	16	6
7	60 ft. × 21 ft.	7/242	2·9	16	8	1895·6484±17·1659	194·21±12·1381	10·2450±0·7030	254·81	13·4418	18	7
8	60 ft. × 24 ft.	8/242	2·5	14	8	2153·0938±21·5100	227·64±15·2099	10·5724±0·7741	291·19	13·5243	20	7
	120 ft. × 12 ft.	8/242	10	29	4	2167·0776±19·8118	213·38±14·0091	9·8464±0·6527	279·29	12·8879	17	7
	240 ft. × 6 ft.	8/242	40	60	2	2165·6083±20·2247	221·55±14·3010	10·2304±0·7251	258·79	11·9500	18	7
9	60 ft. × 27 ft.	9/242	2·2	11	8	2397·7216±25·6513	240·63±18·1382	10·0358±0·7641	303·05	12·6391	17	7
	180 ft. × 9 ft.	9/242	20	39	2	2432·5128±26·2564	231·89±18·5661	9·5329±0·7702	291·41	11·9798	16	7
10	60 ft. × 30 ft.	10/242	2	11	8	2695·0795±29·4388	276·16±20·8164	10·2468±0·7805	353·72	13·1247	18	7
	120 ft. × 15 ft.	10/242	8	22	4	2695·0795±28·5071	267·42±20·1576	9·9225±0·7553	337·85	12·5358	17	7

11	60 ft. × 33 ft.	11/242	1·8	10	8	2956·6813±34·0117	304·21±24·0499	10·2889±0·8220	394·35	13·3376	18	7
12	60 ft. × 36 ft.	12/242	1·7	8	8	3200·4141±41·4225	331·38±29·2901	10·3548±0·9250	400·39	12·5106	19	7
	120 ft. × 18 ft.	12/242	6·7	19	4	3248·1974±36·5184	318·36±25·8224	9·8011±0·8026	408·51	12·5765	17	7
	180 ft. × 12 ft.	12/242	15	29	2	3257·1293±39·7031	302·37±28·0743	9·2833±0·8694	388·43	11·9255	15	6
	240 ft. × 9 ft.	12/242	26·7	39	2	3240·0577±37·9268	334·96±26·8183	10·3381±0·8366	383·33	11·8310	19	7
13	60 ft. × 39 ft.	13/242	1·5	8	8	3497·1406±43·0013	344·01±30·4065	9·8369±0·8779	441·00	12·6103	17	7
14	60 ft. × 42 ft.	14/242	1·4	8	8	3791·2969±47·0963	376·77±33·3021	9·9378±0·8870	487·09	12·8476	17	7
	120 ft. × 21 ft.	14/242	6	16	4	3791·2969±45·1250	361·00±31·9082	9·5218±0·8493	470·12	12·4000	16	7
15	60 ft. × 45 ft.	15/242	1·3	6	8	3939·1146±61·7289	427·67±43·6489	10·8570±1·1211	469·09	11·9085	21	7
	180 ft. × 15 ft.	15/242	12	22	2	4047·4886±57·9324	384·28±40 9644	9·4943±1·0213	466·66	11·5298	16	7
16	60 ft. × 48 ft.	16/242	1·3	6	8	4218·7604±65·4788	463·65±46·3005	10·7532±1·1102	512·82	12·1557	20	7
	120 ft. × 24 ft.	16/242	5	14	4	4306·1875±56·7716	424·84±40·1436	9·8058±0·9413	535·55	12·4366	17	7
	240 ft. × 12 ft.	16/242	20	29	2	4384·1562±58·0822	441·96±41·0350	10·1972±0·9566	512·45	11·8235	18	7
17	60 ft. × 51 ft.	17/242	1·2	6	8	4497·9281±66·9149	463·60±47·3160	10·8070±1·0631	543·07	12·0738	18	7
18	120 ft. × 27 ft.	18/242	4·4	11	4	4795·4432±69·5150	461·11±49·1545	9·6154±1·0345	562·23	11·7243	16	7
	180 ft. × 18 ft.	18/242	10	19	2	4877·0395±73·4036	452·49±51·9042	9·2780±1·0734	565·80	11·6013	15	6
19	120 ft. × 30 ft.	20/242	4	11	4	5390·1591±79·8704	529·80±56·4769	9·8290±1·0579	660·04	12·2453	17	7
	240 ft. × 15 ft.	20/242	16	22	2	5390·1591±84·9267	563·34±60·0522	10·4513±1·1263	621·15	11·5238	19	7
20	120 ft. × 33 ft.	22/242	3·6	10	4	5913·3626±91·7804	580·47±64·8985	9·8163±1·1081	734·48	12·4207	17	7
21	120 ft. × 36 ft.	24/242	3·3	8	4	6400·8281±110·4676	624·90±78·1125	9·7628±1·2320	755·30	11·8000	17	7
	240 ft. × 18 ft.	24/242	13·3	19	2	6496·3947±107·8091	664·58±76·2325	10·2300±1·1867	752·96	11·5904	18	7

Discussion of the results

Plot size and shape.—The relation between the area and the coefficient of variation after eliminating soil heterogeneity (the effect of shape being neglected) is clearly indicated in the table given below :—

Table IV

Relation between the area of plots and coefficient of variation

Serial No.	Area (acre)	Frequency	Average C. V.
1	1/242	1	12·4470
2	2/242	2	11·1472
3	3/242	2	10·3151
4	4/242	3	10·5543
5	5/242	1	10·7307
6	6/242	3	10·0305
7	7/242	1	10·2450
8	8/242	3	10·2164
9	9/242	2	9·7844
10	10/242	2	10·0847
11	11/242	1	10·2889
12	12/242	4	9·9442
13	13/242	1	9·8369
14	14/242	2	9·7298
15	15/242	2	10·1757
16	16/242	3	10·2721
17	17/242	1	10·3070
18	18/242	2	9·4467
19	20/242	2	10·1402
20	22/242	1	9·8163
21	24/242	2	9·9964

It is seen that the variation, with the exception of one or two cases, steadily decreases as the area is increased from 1/242 to 9/242 acre. Commencing with the value 12·4470, the coefficient of variation comes down to 9·7844, which is significantly lower than the starting value. For areas greater than 9/242 acre, the percentage variation fluctuates between 9·4467 and 10·3070 ; there is no significant difference between any two values. Hence it can be concluded that any convenient area beyond 9/242 acre can be taken as the best size of plots suited for sugarcane.

Table V shows the manner in which the coefficient of variation is effected by changing the shape of plots (no consideration being made of the area).

TABLE V

Relation between the shape of plots and coefficient of variation

Ratio of length/width considered	No. of plot sizes considered	Average C. V. (for the several plot sizes)
Between 1 and 2	8	10·3227
Between 2 and 3	3	10·2844
Between 3 and 5	8	10·1304
Between 5 and 10	7	10·0884
Between 10 and 20	9	10·2266
Between 20 and 30	2	9·8505
Between 30 and 80	4	10·3677

There seems to be no difference between the coefficient of variation for the various plot-sizes. But, broadly speaking, the coefficient of variation diminishes until the ratio of length to width is thirty, and beyond this limit it increases. It may be noted that the percentage variation for the class interval 10—20 is 10·2266 which is higher than the preceding and succeeding values. This is due to the inclusion of the coefficient of variation for the area 1/242 acre which is an abnormally high figure. Thus, it can be seen that it is not advisable to increase the ratio of length to breadth of experimental plots above thirty.

The conclusions drawn above are to be taken with a little reserve, for we have not so far taken into account the variation of the coefficient of variation when both the area and the shape of plot change simultaneously. This can be done by making use of Table III. A careful scrutiny of the table indicates that the percentage variation diminishes as the ratio of length to breadth is increased up to a certain limit, keeping the area constant. Similarly when the shape is fixed, the variation can be diminished by increasing the area.

To fix the best size of plots suited for experiments, it is necessary to take into account both the theoretical and the practical aspects. In practice, it is not advisable to have either very small plots or plots of considerable size to secure efficient cultivation and other agricultural operations. The most feasible thing seems to be to strike a golden mean. Any area lying between 9/242 and 15/242 acre, having the shape so adjusted that the ratio of length to breadth lies anywhere between five and fifteen, can be taken as the best size and shape of plots for experimental purposes on canes.

REPLICATIONS

A satisfactory way of determining the number of replications necessary is by using the Student's distribution of Z, (where Z is equal to the deviation from population mean/the standard deviation of the sample), published in "Tables for Statisticians and Biometricians, Part I," page 36. If the deviation, the

standard deviation of the sample and the probability are known, then n can be read directly from the above table. Assuming the difference between the population and sample means to be five per cent or ten per cent of the sample mean, then n for odds 30 to 1 (*i.e.*, for the probability of 0·97 of not exceeding the deviation) can be read from the table. Thus for mean=270·8, standard deviation=33·71, and deviation=ten per cent, n works out to 9.

Summary and conclusions

1. The yields of Co. 210 from 968 plots of 1/242 acre, each 60 ft. × 3 ft., have been analysed with a view to determine the following :—

(*i*) the best size and shape of plots for experiments on canes, (*ii*) the number of replications required to get satisfactory results.

2. The analysis shows that the error of field experiments is partly governed by size and shape of plots. The percentage variation can be diminished by increasing the plot size up to 9/242 or 1/27 acre. Nothing is gained from theoretical aspects by increasing the area beyond 1/27 acre. But for the sake of convenience we can have any area between 1/27 and 15/242 or 1/16 acre. As regards the shape of plots, the investigations indicate that the error can be diminished to a slight extent by increasing the ratio of length to breadth approximately up to a limit of thirty. But in practice it is convenient to adjust the size of plots in such a way as to have the length five to fifteen times the breadth.

3. The number of replications required depends on the percentage variation. If the coefficient of variation is small, it is necessary to have only a few number of repetitions. But if the error is large, it is advisable to repeat the experiment a greater number of times. For land of average variability, six to nine replications will serve the purpose.

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APPENDIX

Tonnage experiment with Co. 210, 1934. New Area, Block—Pusa A.

(Each row is 3 feet apart and the cuts are 60 feet. Area of each plot is 180 square feet; yields in pounds.)

Row No.	Block I				Block II			
	A	B	C	D	E	F	G	H
1	280·5	281·0	313·0	290·0	290·0	264·0	202·0	260·0
2	282·0	286·0	332·5	265·0	282·5	251·0	232·0	243·0
3	251·0	255·0	305·0	299·0	234·5	230·0	224·0	243·0
4	301·0	288·0	286·0	252·5	243·0	207·0	211·5	211·0
5	288·0	255·5	303·0	277·0	226·5	225·0	218·5	243·0
6	310·0	273·5	259·0	248·0	212·0	207·0	216·0	216·0
7	263·5	251·0	272·5	273·0	240·0	261·0	233·0	230·0
8	272·5	284·0	294·5	310·0	261·0	235·0	231·0	233·5
9	296·0	266·5	291·0	301·0	319·0	247·5	232·5	243·5
10	264·5	323·5	271·0	285·0	275·0	214·0	193·0	252·0
11	281·0	281·0	286·0	298·0	276·0	258·0	234·5	265·0
12	320·0	283·0	251·0	261·0	241·5	220·5	211·0	222·5
13	271·0	308·5	272·0	299·0	283·0	239·5	222·0	285·0
14	281·0	292·0	248·5	315·0	230·0	229·0	181·0	215·5
15	284·0	263·0	275·0	310·0	278·5	208·0	204·0	227·0
16	301·0	281·5	270·0	330·0	250·0	249·0	215·5	274·0
17	294·5	278·0	280·5	319·0	254·0	208·0	206·5	208·0
18	311·5	298·5	319·0	306·0	271·0	259·0	202·5	215·5
19	325·0	267·0	288·0	314·0	246·5	226·0	191·5	222·0
20	310·0	283·0	283·5	318·0	268·0	224·0	202·0	233·5
21	297·0	296·0	285·5	301·0	280·0	242·0	214·0	211·0
22	301·0	274·0	291·5	303·5	270·0	257·5	262·0	246·0
23	335·0	272·0	302·0	290·0	260·5	218·5	195·0	200·5
24	314·5	293·0	292·0	318·5	259·5	239·0	215·5	249·5
25	341·5	278·0	307·0	277·0	244·5	207·0	208·0	274·0
26	305·0	297·0	304·0	302·5	265·5	224·0	188·5	280·0
27	337·0	315·0	317·0	294·0	253·0	231·5	223·5	283·0
28	338·0	293·0	289·0	271·5	239·0	188·5	197·5	250·0
29	311·0	306·0	325·0	278·0	252·0	232·0	204·5	262·0
30	270·0	338·0	338·0	261·0	199·5	213·5	197·5	226·0
31	280·0	324·0	311·0	279·0	243·0	221·5	214·0	278·0
32	308·0	306·0	363·0	261·0	270·0	231·0	217·0	273·0
33	304·0	299·0	331·0	280·0	249·5	253·5	248·0	294·0
34	315·0	303·0	306·0	304·0	246·5	264·0	235·0	260·0
35	315·5	339·0	335·5	311·0	275·0	305·0	268·0	283·0
36	342·5	356·0	316·5	296·5	269·0	279·0	283·0	269·0
37	293·0	335·0	343·0	304·0	256·5	270·0	295·0	292·0
38	303·0	325·0	354·0	289·0	290·5	275·0	263·0	287·0
39	307·0	325·0	338·5	305·0	285·5	277·5	299·0	337·0
40	342·0	340·0	317·0	306·0	265·0	269·5	269·5	293·0

APPENDIX—*contd.*

Row No.	Block I				Block II			
	A	B	C	D	E	F	G	H
41	320·0	305·0	316·0	253·5	293·0	265·5	280·0	329·0
42	282·0	255·0	262·0	277·0	224·5	295·0	280·0	262·0
43	304·0	311·0	314·5	279·0	254·5	291·0	249·0	286·5
	Block III				Block IV			
44	326·0	329·0	333·0	306·5	266·0	275·0	265·0	257·0
45	269·0	257·0	295·5	312·0	273·0	246·0	248·0	266·0
46	255·0	300·0	325·0	256·0	244·5	265·0	217·0	244·5
47	288·0	287·0	261·0	244·0	255·5	225·5	206·5	248·0
48	284·5	281·0	272·0	258·5	244·0	247·5	224·5	299·0
49	304·5	308·0	303·5	263·0	259·5	281·0	247·0	280·0
50	295·5	286·0	325·0	267.0	254·0	253·5	238·0	260·0
51	303·0	292·0	295·0	231·5	277·5	268·0	235·0	272·0
52	304·0	258·0	316·0	225·0	261·5	270·5	238·0	283·5
53	264·0	302·0	273·0	228·0	250·0	252·0	222·0	295·0
54	309·0	274·5	306·5	235·0	242·0	255·0	225·0	256·0
55	297·0	303·0	328·5	276·0	232·0	257·0	266·5	248·0
56	296·0	294·5	329·5	237·5	225·5	238·0	242·0	240·0
57	319·0	295·5	311·0	256·0	234·0	249·0	261·0	241·0
58	310·0	270·0	325·0	262·5	245·5	252·5	238·5	260·0
59	294·0	310·0	283·0	283·5	236·5	239·0	241·0	231·0
60	295·0	278·0	280·0	300·0	267·0	254·5	242·0	241·0
61	296·0	304·5	329·5	294·5	250·0	233·0	221·0	239·5
62	293·0	315·0	288·5	316·0	271·0	241·0	237·5	292·0
63	292·0	296·0	276·0	313·0	245·0	300·0	267·5	300·5
64	347·0	312·5	276·5	285·0	230·0	289·0	255·5	264·0
65	262·0	298·5	262·0	300·0	255·0	275·5	256·0	237·0
66	323·5	354·5	266·5	309·5	289·0	299·0	246·0	277·0
67	291·0	279·0	287·0	253·0	281·5	198·0	214·0	281·0
68	306·5	294·0	324·5	344·0	291·0	240·0	255·5	267·0
69	353·0	298·0	287 0	365·5	304·5	267·0	276·0	289·0
70	280·0	262·5	278·0	378·5	339·5	278·0	262·5	335·0
71	296·0	294·0	304·0	316·5	299·5	212·0	265·0	276·0
72	302·0	333·0	269·5	354·0	328·0	303·0	247·5	280·0
73	314·0	332·5	284·0	356·5	311·0	286·5	227·5	269·0
74	336·0	404·5	350·5	350·0	306·5	307·5	226·5	251·0
75	320·0	371·5	334·0	383·0	325·5	285·0	256·5	256·0
76	296·0	333·0	381·5	351·0	272·0	291·0	260·5	227·0
77	324·5	311·0	355·5	316·5	296·0	328·0	259·0	312·5
78	302·5	336·0	284·0	279·0	298·0	327·0	243·0	241·0
79	314·5	317·5	261·0	323·0	296·5	289·5	239·0	222·0
80	290·0	366·5	262·0	359·0	330·0	365·0	257·0	249·0
81	360·0	313·5	310·5	380·0	262·5	360·5	253·5	228·0
82	352·0	371·0	304·0	380·0	310·0	387·0	277·0	254·0
83	345·0	342·5	309·0	347·0	341·5	354·0	288·5	260·5
84	347·0	404·0	263·0	299·0	296·0	291·5	292·0	287·0
85	327·0	367·0	292·0	376·0	357·5	405·0	288·5	304·0
86	286·5	361·0	332·0	369·0	327·0	348·0	308·0	317·5
87	293·0	320·0	306·5	299·5	332·5	294·0	269·0	274·5

APPENDIX —*concld.*

Row No.	Block V				Block VI			
	A	B	C	D	E	F	G	H
88	250·0	277·0	308·5	241·0	247·5	303·5	233·0	286·0
89	221·0	232·5	277·5	317·5	296·0	327·5	279·0	297·5
90	185·0	245·0	306·0	280·0	278·0	279·5	247·0	282·0
91	155·5	205·0	243·0	261·0	240·0	307·0	227·0	274·5
92	250·5	200·0	243·5	271·0	269·5	274·0	218·0	291·0
93	205·0	220·5	276·5	282·5	286·5	290·0	226·0	261·5
94	257·0	232·5	275·5	255·5	291·5	297·0	247·0	297·5
95	211·0	210·5	267·0	279·0	289·5	282·5	258·5	254·0
96	176·0	186·0	262·0	285·0	273·5	278·5	234·5	255·0
97	175·5	183·0	220·5	272·0	257·0	255·5	226·0	225·0
98	214·5	188·0	239·0	254·5	273·0	235·5	227·0	248·5
99	186·0	160·0	214·0	269·0	263·0	269·5	213·0	268·5
100	188·5	164·0	206·5	251·5	250·0	268·0	258·0	262·0
101	181·5	165·5	223·5	301·0	230·0	282·5	245·0	235·0
102	191·0	186·0	207·0	259·0	257·0	292·5	262·5	208·0
103	191·5	182·0	221·0	230·0	230·0	273·0	256·5	243·0
104	188·5	189·5	211·5	249·0	266·5	276·0	243·0	301·5
105	194·5	204·0	201·0	234·0	227·0	281·0	224·0	287·0
106	233·5	222·5	191·0	236·0	235·0	262·5	234·5	295·0
107	203·5	210·0	219·0	236·5	266·0	264·0	271·0	298·5
108	232·5	236·0	186·0	210·5	235·0	300·0	225·0	266·0
109	203·5	216·5	215·0	228·5	266·5	312·0	251·0	322·5
110	202·0	222·0	206·0	255·5	227·5	324·5	212·5	247·0
111	206·0	198·5	189·0	258·5	258·5	275·5	216·0	264·5
112	192·5	219·0	218·0	343·0	241·0	283·5	249·5	260·0
113	226·5	270·5	231·0	327·5	252·0	283·0	277·0	285·0
114	174·5	210·0	246·5	343·0	289·5	276·0	204·0	270·5
115	201·5	212·0	195·0	310·5	361·0	313·0	231·5	301·5
116	216·0	262·5	225·5	274·0	407·0	303·5	208·5	234·5
117	182·5	231·5	227·5	312·0	315·0	322·0	219·0	279·0
118	212·5	259·5	219·0	244·0	317·0	273·0	223·5	265·5
119	204·0	255·0	245·0	272·5	266·5	211·0	240·0	271·5
120	244·0	222·5	239·0	259·5	211·5	212·5	237·5	255·5
121	222·0	255·5	271·5	311·0	243·0	244·0	244·5	319·0

THE CLASSIFICATION OF THE AUTUMN RICES OF THE PUNJAB AND WESTERN UNITED PROVINCES

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CONTENTS

		Page
I.	Introduction	930
II.	Material and method	932
III.	Literature	933
IV.	Bases of classification	934
V.	Key to the classification	934
VI.	Summary	936
	References	937

I. Introduction

The total amount of annual precipitation and its distribution largely determines the extent to which rice is cultivated in India. Wherever the rainfall is abundant and fairly uniform in distribution, rice is a crop of primary importance, but in areas such as the Punjab and the Western United Provinces where the monsoon is less bountiful and reliable, rice cultivation is relatively unimportant. Of the total paddy area in British India, Punjab and the Western United Provinces together claim a little over two per cent. The distribution of this is given in Table I.

TABLE I

Area under rice in the districts of the Punjab, 1933-34

District	Area
Hissar	7,775
Rohtak	455
Gurgaon	18
Karnal *	66,350
Ambala *	57,684
Simla	1,051
Kangra *	116,500
Hoshiarpore *	35,024
Jullundur	2,091
Ludhiana	1,281
Ferozepore	24,186
Lahore	31,978
Amritsar	34,768
Gurdaspur *	75,506
Gujranwala *	164,758
Sheikhupura *	142,794
Gujrat	20,235
Shahpur	5,866
Jhelum	1,134
Rawalpindi	995
Attock	14
Mianwali	1
Montgomery	28,109
Lyallpur	2,506
Jhung	2,270
Multan	32,897
Muzaffargarh	37,188
Dera Ghazi Khan *	69,960
Sialkot *	71,392

Area under rice in some districts of the United Provinces, 1933-34

District	Area
Dehra Dun	24,898
Saharanpore *	106,594
Muzaffarnagar *	36,610
Meerut	17,830
Bulandshahr	2,230
Bareilly *	161,235
Bijnor *	153,689
Badaun	39,378
Moradabad *	119,154
Pilibhit *	121,426
Aligarh	1,625
Muttra	10
Agra	25
Mainpuri	26,130
Etah	11,377

It is only in the districts marked * that the cultivation of rice is somewhat important. The staple food of the people in these and other districts in the area under consideration, as a whole, is wheat, and rice forms a regular article of diet only in the case of upper classes.

The climatic conditions prevailing in these parts do not favour the successful growth of winter or long duration paddy varieties. In contrast, therefore, to the case of Bengal and Bihar, early maturing types are almost invariably grown, the crop being harvested before the onset of cold weather, *i.e.*, about the end of October, and the land then sown with a *Rabi* crop.

It will thus be seen that the rice types evolved at Karnal are very different from those classified at Pusa. It is in this respect that the paddy classification described in these pages, differs from the original one. Although the basis of the classification evolved by the senior author remains the same and has been adhered to in entirety, the inclusion of autumn paddy types forms an important supplementary work. Besides, it proves the applicability of the Pusa scheme of classification of rices, to a group of paddy types which are quite distinct from those worked on at Pusa.

II. Material and method

One hundred and fifty-seven paddy samples were collected from the important rice-growing districts of the Punjab and the Western United Provinces. The types isolated in the United Provinces by Sethi [1931] were also kindly supplied by him, but with the exception of a few samples from Dehra Dun, these proved to be late and unsuitable for Karnal conditions.

After eliminating duplicates, forty-one distinct types were isolated from the original collection of 157 kinds. Since the present work is a continuation of that commenced by the senior author at Pusa, where 123 strains were isolated and described [Kashi Ram and Chetty, 1934], the types described in the present paper are numbered from 124 to 164. The localities whence the latter were obtained are enumerated in Table II.

Table II

Types isolated from several districts of the Punjab and the United Provinces

Districts	Types isolated
The Punjab—	
Ambala	143, 145, 155
Ferozepore	139, 151
Gujrat	136, 141, 159

TABLE II—*contd.*

Districts	Types isolated
Hissar	128, 133, 157, 158, 161
Karnal	124, 126, 129, 130, 132, 134, 144, 147, 148, 150, 152, 153, 154, 162, 164
Muzaffargarh . . .	142
Sheikhupura	125, 127, 131, 135, 137, 138, 140, 146, 156, 160, 163
United Provinces—	
Dehra Dun	149

III. LITERATURE

The multiplicity of rice varieties and the high degree of physiological adaptation displayed by the paddy plant are probably responsible for the lack of uniformity in methods of classification. Kikkawa [1912], Hector [1916, 1922, 1929], Graham [1913], Thadani and Datta [1928], Sethi and Saxena [1930], etc., have all made contributions to the classification of rices ; these will not be discussed here as they have been recently reviewed [Kashi Ram and Chetty, 1934]. It may be said, however, that two distinct kinds of classificatory schemes have emerged ; those based on morphological characters and those based on both morphological and agricultural characters. Thus Koernicke [1885] used the character of the grain for separating the glutinous rices from the common starchy ones. Kikkawa [1912] employed both morphological and agricultural characters for sorting out the different varieties. On the other hand, Hector [1930] pointed out that the so-called "*Aus* —High Land rice, *Aus*—Low Land rice, *Aman*—transplanted rice and *Aman*—Low Land rice" are difficult to distinguish from each other morphologically, and yet are clearly different as regards their requirements of water, etc. But in Graham's opinion [Graham, 1913] the agricultural characters are less constant than the morphological ones and therefore not sufficiently dependable for use as bases of classification. He mentions that while early, medium, and late rices from Jubbulpore matured, on the average, in 113, 120 and 128 days respectively, rices of the same classes from Raipur matured in 124, 127 and 138 days respectively.

In the present paper, the scheme of classification, based on morphological characters, evolved at Pusa [Kashi Ram and Chetty, 1934] has been adhered to.

IV. Bases of classification

The Pusa classification referred to in the last paragraph of Section III is based on morphological characters, such as the constitution of the rice kernel, presence or absence of clustering in spikelets, length of the outer glumes, and the presence or absence of elongated internodes with aerial roots. After these, the colour of the inner glumes was chosen as the most reliable criterion. Furthermore the inner glumes exhibit a wide range of easily distinguished colours in different varieties and hence afford a ready means of discrimination. Thereafter the colour of the apiculus, outer glumes, kernel, and internode was used, and finally the grain size and shape, which are the most constant of the quantitative characters, were used in separating the ultimate classes.

V. Key to the classification

Starchy rice			
Single grained			
Short outer glumes			
Solitary spikelets			
Short internode			
Inner glume—straw			
Apiculus—colourless			
Outer glumes—white			
Kernel—white			
Internode—green			
Grain size—IIb	Slender	124	Beardless, stigma—white
	Medium	125	Beardless, stigma—light purple.
IIc	Very slender	126	Bearded, stigma—light purple.
	Medium	127	Beardless, stigma—white.
IIIc	Slender	128	Beardless, scented.
		129	Tipped.
		130	Tipped later than 129.
		131	Beardless, narrow leafed.
Apiculus—brown			
Outer glumes—white.			
Kernel—white			
Internode—green			
Grain size—IIb	Bold	132	Tipped.
	Medium	133	Bearded.
Outer gulmes—brown			
Kernel—white			
Internode—green			
Grain size—IIc	Medium	134	Beardless, later than 135.
IIIc	Medium	135	Tipped.
		136	Bearded.
Apiculus—purple (restricted)			

Outer glumes—white		
Kernel—white		
Internode—green		
Grain size—IIc	Slender	137 Leaf sheath purple.
Kernel—light red		
Internode—green		
Grain size—IIb	Medium	138 Beardless.
IIIc	Slender	139 Beardless.
Apiculus—purple (spread)		
Outer glumes—purple		
Kernel—white		
Internode—green		
Grain size—IIb	Medium	140 Bearded, stigma white.
IIIc	Medium	141 Bearded, stigma light purple.
	Bold	142 Bearded.
Inner glumes—olive	uniform	
Apiculus—brown		
Outer glumes—white		
Kernel—white		
Internode—green		
Grain size—IIc	Slender	143 Beardless, scented.
	Bold	144 Bearded.
IIIc	Slender	145 Beardless.
Kernel—red		
Internode—green		
Grain size—IIb	Medium	146 Tipped.
Apiculus—brown		
Outer glumes—white		
Kernel—white		
Internode—green		
Grain size—IIc	Slender	147 Beardless.
IIIc	Medium	148 Tipped.
Outer glumes—purple		
Kernel—white		
Internode—light gold		
Grain size—IIb	Medium	149 Beardless, dwarf.
Apiculus—purple		
Outer glumes—purple		
Kernel—white		
Internode—green		
Grain size—IIb	Medium	150 Beardless.
	Bold	151 Tipped.

Inner glumes—olive furrowed on green base
Apiculus—colourless
Outer glumes—white
Kernel—white
Internode—green
Grain size—IIb Bold 152 Beardless.
Kernel—red
Internode—green
Grain size—IIc Medium 153 Beardless.
Inner glumes—brown
Apiculus—brown
Outer glumes—brown
Kernel—white
Internode—green
Grain size—IIc Slender 154 Beardless.
Medium 155 Tipped.
156 Bearded.
IIIc Medium 157 Tipped.
Inner glumes—gold
Apiculus—colourless
Outer glumes—white
Kernel—white
Internode—gold
Grain size—IIb Medium 158 Tipped.
Slender 159 Beardless.
Kernel—light red
Internode—gold
Grain size—IIb Medium 160
IIc Slender 161
Inner glumes—dark gold
Apiculus—colourless
Outer glumes—white
Kernel—white
Internode—gold
Grain size—IIb Medium 162 Beardless.
IIc Medium 163 Beardless.
164 Bearded.

VI. Summary

1. Forty-one types have been isolated from a collection of samples from the important rice-growing districts of the tract under consideration. As late varieties were unsuited to the conditions prevailing at Karnal, the work was limited to the autumn rices.

2. The classifications of previous investigators are briefly referred to. The classification of Kashi Ram and Chetty [1934] is followed in this paper.

3. A key to the classification is given.

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INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET

IX. DIMPLED GRAINS*

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(With Plate LIII and one text-figure)

In the varietal collections of sorghum at the Millets Breeding Station, Coimbatore, there is a small group represented by a variety called *Sakkara gulige jonna* (Sugar Pill Sorghum) which comes from the district of Bellary in the Madras Presidency. This sorghum has its grains "dimpled". This dimple is in the nature of a depression towards the top of the grain, and is easily visible where it occurs. The sorghum grain is asymmetrical in the disposition of its endosperm, there being a bump on the side away from the embryo. This bump is towards the outermost glume. The dimple when it occurs is on the bumpy side, and always away from the embryo. The dimple can occur in grains having either a floury or corneous endosperm. When the grains are cut there is always a small hollow (varying in its size) immediately behind the dimple.

Plants having this type of grains are not usually vigorous. In the Bellary district, varieties with this type of grain are not grown on an extensive scale, but select cultivators treasure this variety and grow it in small patches to give them the soft and tasty grains that are consumed in the dough stage. But for this careful preservation, these delicate varieties which are probably mutational in origin, would not have survived.

White, yellow and red types were collected from the Bellary tract. By crossing with a brown-grained variety, a dimpled brown type was fixed. Subsequent to this similar dimpled types have been obtained from Bombay and the Central Provinces. This dimpling occurred in both pearly and chalky grains

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[Rangaswami Ayyangar *et al.*, 1934]. Spotted as these peculiar varieties were in an area predominantly *Sorghum cernuum* Host in distribution, the important types partake of *cernuum* characteristics. For the sake of easy identification and future work on this characteristic type of grain, this variety has been designated *S. cernuum* Host var. *areolatus* Rangaswami.

An examination was made of the manifestation of this dimpling on the ear-head in pure varieties. Taking all degrees of dimpling into consideration, dimpling may be said to occur in practically every grain, the maximum number of grains that did not show the dimpling being less than ten per cent. As will be explained later, this ten per cent could be accounted for as resulting partly from crosses occurring in nature and partly owing to the difficulty of spotting minute dimples.

The progress of dimpling was watched in thirty-three ear-heads of six differing varieties. It takes forty to forty-five days for a grain to ripen from the time of pollination. Young grains in the early milky stage begin to manifest dimpling even when they are twelve days old. In another fifteen days dimpling ceases. It is at the stage when dimpling is nearing completion that the grains are most prized for eating.

Crosses (reciprocal) were made between dimpled and normal (non-dimpled) grain types. In all cases where the non-dimpled grain type was used as the mother parent, the crossed grains were also non-dimpled. In the reciprocal crosses with the dimpled grain type as the mother parent, all the crossed grains were non-dimpled.

First generation plants were raised from these crosses. It was noted that the grains in the ear-heads raised, consisted of a mixture of both dimpled and non-dimpled grains. Counts taken in forty-nine hybrid ear-heads are presented in Table I.

TABLE I

Counts of dimpled and non-dimpled grains in hybrid ear-heads from eight artificial crosses

Head No.	No. of grains		Percentage of dimpled grains
	Non-dimpled	Dimpled	
1	2159	716	24·9
2	911	284	24·0
3	1268	401	24·0
4	3037	916	23·2
5	1087	318	23·0
6	1706	510	23·0

TABLE I—*contd.*

Head No.	No. of grains		Percentage of dimpled grains
	Non-dimpled	Dimpled	
7	1934	569	22·7
8	2110	613	22·5
9	2029	582	22·3
10	2683	770	22·3
11	2019	578	22·2
12	1468	416	22·1
13	1623	460	22·1
14	1195	337	22·0
15	1648	464	22·0
16	2085	590	22·0
17	2933	822	21·9
18	2285	636	21·8
19	1716	471	21·5
20	2211	597	21·3
21	2736	733	21·1
22	1579	425	21·0
23	2397	635	20·9
24	1738	458	20·9
25	2392	628	20·8
26	2530	655	20·6
27	2833	732	20·5
28	1617	415	20·4
29	1910	487	20·3
30	2193	553	20·1
31	1464	378	20·0
32	1971	489	19·9
33	2340	573	19·7
34	2081	509	19·7
35	1497	366	19·7
36	1504	366	19·6
37	2240	542	19·5
38	2190	532	19·5
39	2353	572	19·5
40	1332	322	19·5
41	2440	589	19·4
42	1466	351	19·3
43	1638	385	19·0
44	1944	457	19·0
45	2093	488	18·9
46	2931	682	18·9
47	2629	606	18·7
48	2609	597	18·6
49	2956	675	18·6
Total	99710	26250	20·8

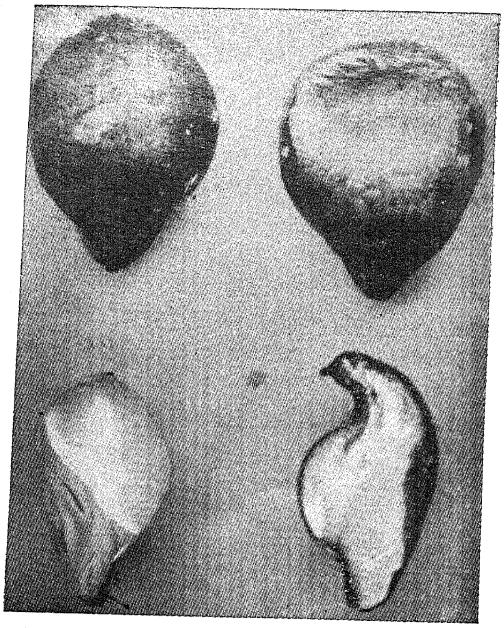

FIG. 1. Top : Non-dimpled and Dimpled grains—(Front view).
Bottom : Non-dimpled and Dimpled grains—(Side view, in section).

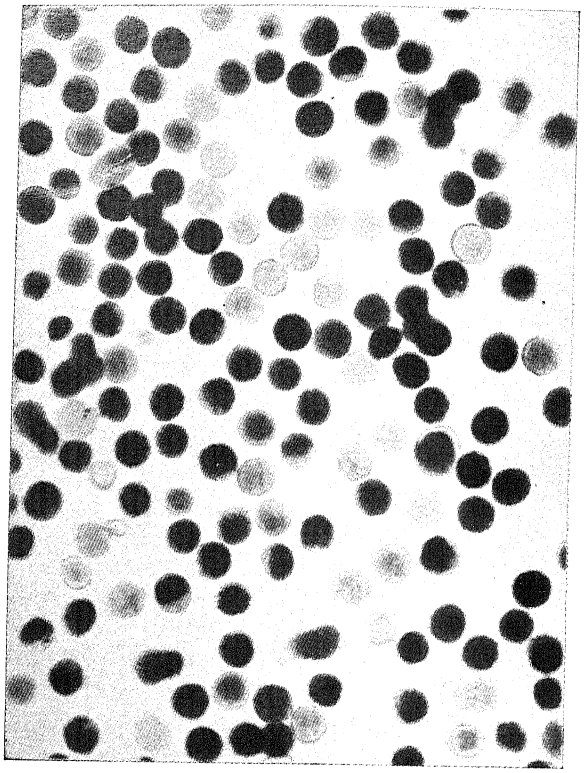

FIG. 2. Pollen dimorphism—Deep and light stained grains from hybrid earhead.

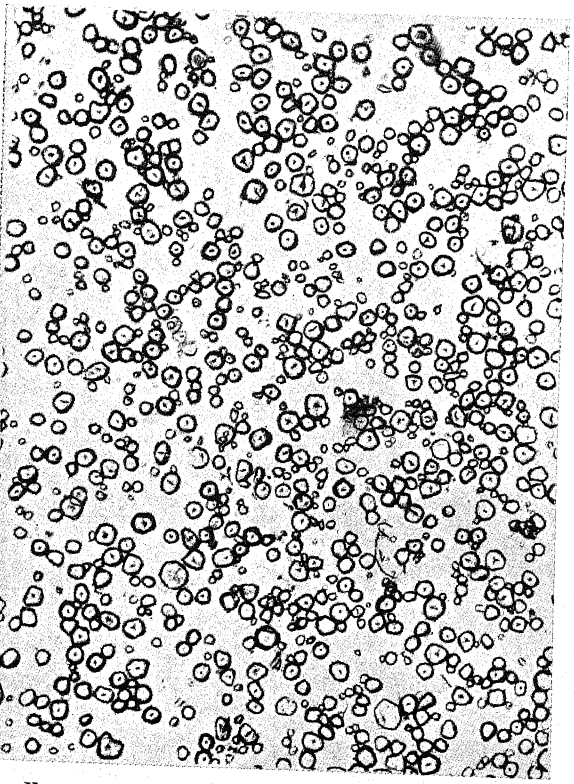

FIG. 3. Starch grains—Non-dimpled seed.

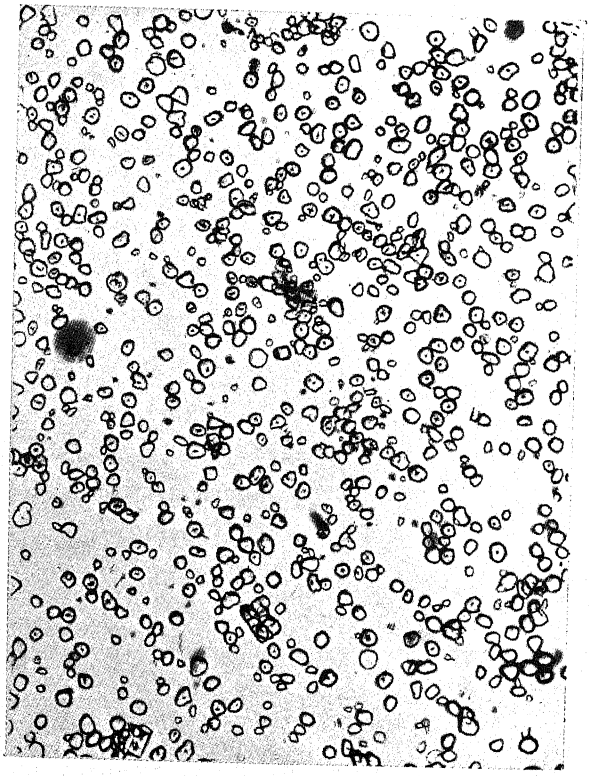

FIG. 4. Starch grains—Dimpled seed.

The above numbers give an approximate 3 : 1 ratio, the non-dimpled grains being in excess. A practical difficulty is experienced in the sorting of the grains which might possibly explain the shortage in the dimpled group. Cases of very faint dimpling run the risk of being classed in the non-dimpled group and thus swell their numbers. That this does occur was proved later on when stray non-dimpled grains gave rise to plants with all grains dimpled, proving that dimpling might fluctuate towards very near non-dimpling and thus escape notice. This practical disability might not altogether explain the aberration in numbers. It is quite possible as in maize [Correns, 1902] that the pollen of dimpled varieties suffers from a handicap in competitive fertilization.

That the ratio obtained in the above table (Table I) is an undoubted 3 : 1, will be obvious from the behaviour of the " 3 " group [Rangaswami Ayyangar, 1934]. In this group, out of the 103 plants obtained from non-dimpled seeds, thirty-four gave heads with all grains non-dimpled and sixty-nine gave hybrids having both non-dimpled and dimpled grains.

The starch grains in non-dimpled and dimpled varieties were examined. The grains are simple and are polygonal in shape. In the non-dimpled varieties the starch grains were larger than in the dimpled varieties. Photographs of starch grains in the dimpled and non-dimpled varieties show this difference clearly (Plate LIII, figs. 3 and 4). Three hundred starch grains in each of the non-dimpled (*A. S.* 158) and dimpled (*A. S.* 219) varieties were measured and the measurements plotted (Fig. 1). It will be seen that the mode for non-dimpled starch grains is at about 26μ, whereas the mode for the dimpled starch grains is about 15μ. Analysis of the dimpled grains, kindly done by the Agricultural Chemist, shows that the amount of reducing sugars in the dimpled grains is thrice the quantity of that contained in the non-dimpled grains, the non-reducing sugars being equal in both.

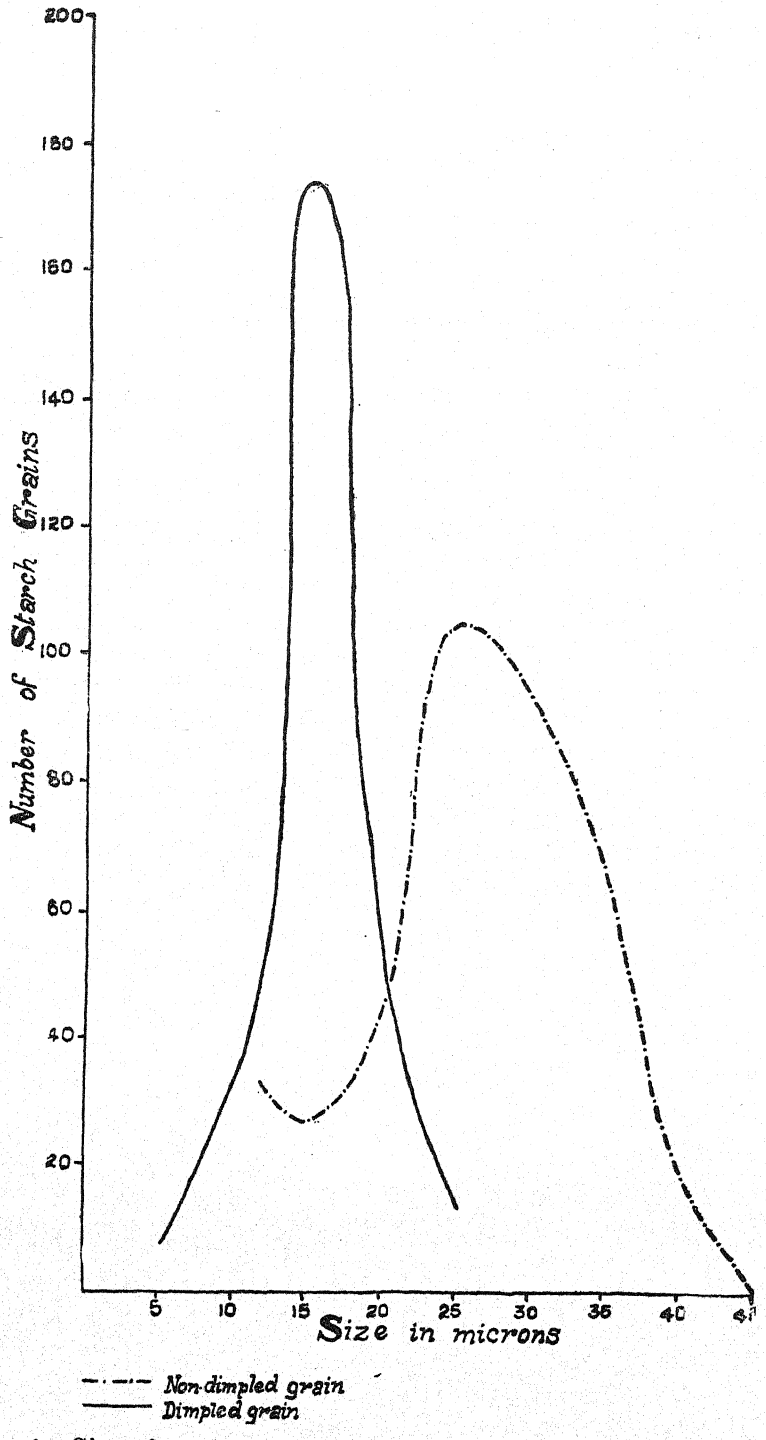

Fig. 1. Size of starch grains from dimpled and non-dimpled seeds.

The dimpled varieties are few in number. The majority of sorghums have non-dimpled seeds. Starch grains in typical non-dimpled varieties and species of sorghum were examined and it was noticed that in all these the starch grains were large and were about 25μ in diameter.

This examination leads one to the conclusion that the starch formation in the dimpled grains is arrested at some stage in their growth, leading to the non-development of the grains to fullness. As the grain ripens and loses moisture, the imperfect development of the starch grains leads to an unfilled cavity inside and the dimple outside.

The Mendelian theory of inheritance postulates random assortment and fertilisation among the gametes. Parnell [1921] first demonstrated that, in rice plants heterozygous for the glutinous character, two types of pollen grains, one staining red when treated with iodine and the other blue, were produced in approximately equal numbers. Demerec [1924], Brink and MacGillivray [1924] in maize, Longley [1924] in *Coix*, and Karper [1933] in sorghum, noted a similar differentiation between the starchy and waxy pollen. The type of dimorphism in pollen, reported herein, belongs to a different category. When treated with iodine the staining was of two shades of blue—light and deep.

An examination of the pollen of dimpled and non-dimpled pure lines was made. Pollen from the dimpled variety (*A.S.* 219) when treated with one per cent iodine stained light blue. In these varieties, the contents of the pollen grains could be made out due to the light stain. The pollen from the normal non-dimpled variety (*A. S.* 158) stained very deep blue in iodine. The whole grain was so intensely blue-coloured that the contents inside the grain could not be made out. Plants heterozygous for dimpling were found to have both kinds of pollen grains, those in which the starch reserve was stained light and those in which it stained deep blue, in about equal proportions on the same head (Plate LIII, fig. 2). The photograph clearly shows the difference in reaction to the staining reagent by the two types of pollen. Counts of pollen grains from ten heterozygous plants gave the expected 1 : 1 ratio.

TABLE II

Pollen counts in hybrid ear-heads

(Cross No. A. S. CLXXVII)

Plant No	Total No. of pollen grains	Deep stained pollen	Light stained pollen
1	2,233	1,103	1,130
2	2,394	1,181	1,213
3	2,190	1,096	1,094
4	2,317	1,172	1,145
5	1,181	577	604
6	1,788	919	869
7	1,458	727	731
8	1,673	826	847
9	1,666	858	808
10	1,573	813	760
Total	18,473	9,272	9,201

This dimorphism results in the production of both dimpled and non-dimpled grains in the same ear-head—a clear and easily spotted instance of xenia in sorghum.

The female gametophyte, the embryo-sac, was also examined. The technique adopted was that of Brink [1925] for maize. In the embryo-sac of the dimpled type, a light bluish stain was observed. Non-dimpled embryo-sacs gave the deep blue reaction. Out of the forty-six sacs examined twenty-four gave a deep blue reaction, twenty light blue, and two were doubtful. Under the difficulties of the technique involved in this examination, the almost equal numbers in the two groups are undoubted in their import. The gene resulting in dimpling, it is obvious, exerts the same influence in the female gametophyte as in the pollen. It will thus be seen that there is both pollen and embryo-sac dimorphism manifested in this cross between non-dimpled and dimpled varieties. A factor **dp** is responsible for an arrest in the development of starch grains with the resultant dimple in the mature grain. Factor **Dp** produces the normal well-filled grains of sorghum with the usual size in starch grains. Dimpled grains (**dp**) are a simple recessive to non-dimpled grains (**Dp**).

SUMMARY

Grains with a dimple on them occur in sorghum. Varieties with such grains are rare. Dimpling has been noted in white, yellow, red and brown grains, and in grains that are pearly or chalky in appearance. Dimpling could occur in grains with floury or corneous endosperm. Grains from dimpled varieties are consumed as a delicacy in the dough stage when they are sweet. Dimpled grains contain three

times the amount of reducing sugars found in non-dimpled grains. Dimpling results from the smallness of the starch grains. This smallness is due to the arrest in the development of the starch grains. The pollen grains in dimpled varieties stain light blue and in the common varieties deep blue with iodine. In hybrids between dimpled and common non-dimpled varieties, gametic dimorphism, in both pollen grains and embryo-sacs has been noted. Non-dimpled grains in ear-heads with dimpled grains betray xenia. Dimpled grain occurs frequently in the group *Sorghum cernuum* Host and the dimpled variety has been named *Sorghum cernuum* Host var. *areolatus* Rangaswami. Dimpled grains (**dp**) are recessive to normal non-dimpled (**Dp**) grains.

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THE STORAGE OF FUNGAL CULTURES

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(With one text-figure)

INTRODUCTION

The prolonged storage of cultures of fungi presents many difficulties, particularly in hot climates. A temperature not exceeding 25°C. is generally admitted to be desirable, the optimum storage temperature varying from 0° to 20°C. according to the species under consideration. The use of a cellar as culture store may be sufficient to secure this, but if refrigeration is needed, it is desirable to avoid a system which involves either air circulation or an increased humidity of the atmosphere, since both these factors lead to infected cultures.

A damp storage atmosphere increases the risk of infection both by mould fungi and by mites. An atmosphere that is too dry, on the other hand, desiccates the culture media, and necessitates frequent sub-culturing. To try and check evaporation by the use of rubber caps for the tubes is to invite infection, since such caps merely cause the cotton plugs to take up moisture from the medium without excluding atmospheric infection. A better plan, adopted in some culture collections, is to dip the plugged ends of the tubes in melted paraffin wax. The only objection to this method is that the cultures are cut off from the external air supply, whilst vegetative growth still continues, and are thus forced to live amid the accumulation of their own toxic products. Such cultures consequently are liable to abnormal development or early death.

If ordinary cotton plugs are employed, the most serious danger is that of infestation by mites. Many workers are apt to overlook this peril until they have once had experience of it. Mite infestation is not readily noticeable in its early stages. Later, cultures acquire a somewhat moth-eaten or glazed appearance, and mites can be seen on careful examination of the tube under a low power of the microscope. Mites travel from tube to tube, undeterred by the cotton plugs, and leaving bacterial and fungal spores behind them as contaminants. Infection by small-spored fungi, *e.g.*, of the *Cephalosporium* type, is a common indication that mites are at work. A preference is often shown for certain types of culture; *Penicillium* spp. seem to be particularly liable to attack.

As precautionary measures, volatile repellents (*e.g.*, naphthalene) suffer from the objection that they interfere with normal fungal growth. In the writer's experience both pyridine and carbon tetrachloride have been found fairly harmless if their vapour is not too concentrated; also the occasional application to the plugs of a few drops of one per cent naphthalene in alcohol, or of petrol ether. Some workers [Thom, 1930] dip the plugged ends into a solution containing mercuric chloride. The best course, however, is to avoid mite infection altogether by keeping shelves and incubators clean, by excluding infected material, and by never allowing tubes to lie with their plugs touching the working bench.

Methods of storage other than on agar slope cultures have been tried by various investigators. Fragments of mycelium—with or without agar medium—are said to remain viable if kept in sugar solution. Spores have been preserved for long periods mixed with earth, chopped straw, chalk, or sand. Thaysen [1924] described a method of storage of bacteria on sand in sealed tubes, and in a later paper [Thaysen, 1934] records further data on the viability of both bacterial and fungal cultures preserved by this method. The only fungi recorded are *Penicillium "glaucum"*, *Aspergillus glaucus* and *Rhizopus japonicus*. All were healthy after twelve months storage on sand. After twelve years' storage, spores of the first two species were non-viable, but those of *Rhizopus japonicus* gave rise to an active culture.

Experimental

Sand cultures

Method of preparation.—Purified sand suitable for this purpose may be obtained from most chemical firms, or fine "silver sand" may be purified in the laboratory by warming with hydrochloric acid, washing until free from acid, and then heating in a muffle furnace. Test tubes—preferably rather narrower than the normal variety—are then filled with the treated sand to a height of about one inch from the bottom, plugged with cotton wool, and sterilised for two hours at 160°C.

An appreciable quantity of spores from a healthy culture of the fungus to be preserved is removed by means of a wire loop, and mixed with the sand, the usual precautions being taken to avoid infection during this transfer. Spores are in some cases more easily picked up if the loop is first moistened with sterile water.

The inoculated tubes are incubated at 25°—30°C. in a dry atmosphere for some days in order that the spores may dry off, and are then sealed in the blow-pipe. The worker unaccustomed to glass blowing requires a little practice before he can carry out the sealing process neatly and without failures. The following hints may be useful:—

(1) Employ good quality glassware, not too thin.

(2) Use a foot-blower and a fairly small flame.

(3) Soften thoroughly a small length of the tube and then pull out sharply without, however, sealing the tube (stage 2 in Fig. 1).

(4) Re-soften and seal at the tapering portion, at the same time warming the upper part of the enclosed air space so that the sealed end remains nicely rounded, without either excessive expansion or excessive shrinkage (stage 3 in Fig. 1).

(5) Transfer to the cool flame at the critical moment, then set the tube aside to cool slowly, taking care that the sand is not allowed to come into contact with the hot part of the tube.

The ideal to aim at is to produce a seal indistinguishable from the original end of the tube. Such sealed cultures are very robust, and convenient to handle or to send through the post.

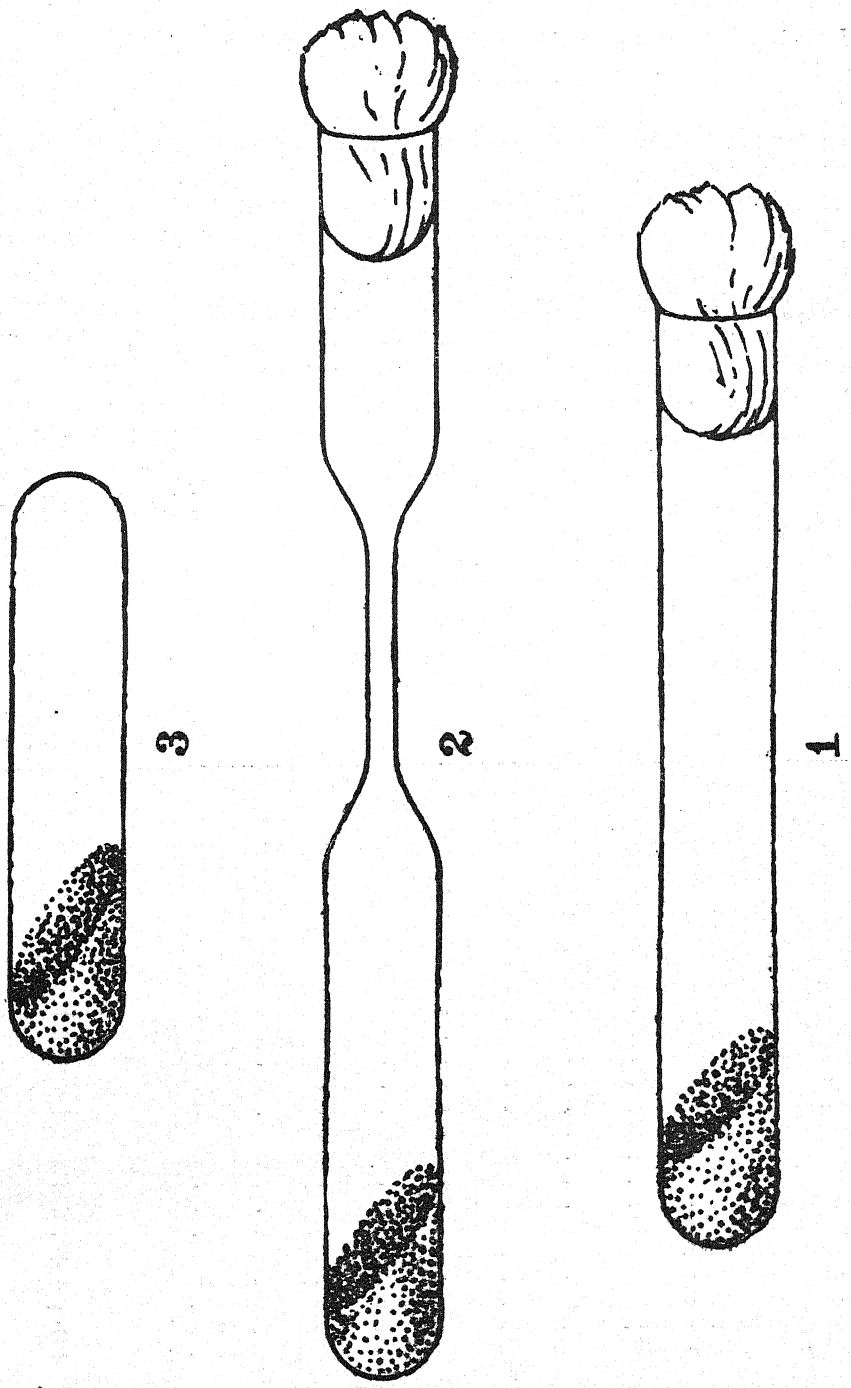

Fig. 1.

Method of sub-culturing.—When it is desired to open a sealed tube, a small file mark is made near the seal. The end of a thin glass rod is then heated to redness in a Bunsen or blow-pipe flame, and pressed firmly against the file mark, with the rod normal to the surface of the glass. The tube should crack all round, and after passing through the flame to sterilise the outer surface, the top may be removed by a gentle tap. The sand is then tipped into a sterile Petri dish, and 20 c.c. of a suitable agar medium, melted and cooled to 42°-45°C. is poured over it. An alternative method that is to be preferred is to tip the sand into a tube of suitable liquid nutrient medium ; about one c.c. of the liquid is then used for plating as before, and the remainder of the liquid, containing the sand, is incubated as a reserve culture in case of failure of the plate culture.

Experimental.—In 1931, sand cultures were made in England of a number of representative mould fungi isolated principally from textiles [Galloway, 1930]. These cultures were opened after two years' storage at normal English laboratory temperatures, and the results are shown in Table I. Authorities for species are omitted for the sake of brevity.

TABLE I

Sand cultures

Species	Viability after two years (or 18 months where indicated with asterisk)
Aspergillus chevalieri, strain A	+
" " " B	+
A. amstelodami	+
A. repens	+
A. nidulans, strain A	+
" " " B	+
A. versicolor, strain A	+
" " " B	+*slow**
" " " C	—*
A. niger, strain A	+
" " " B	+
A. candidus, strain A	+*
" " " B	+*
" " " C	+
A. flavipes	+
A. effusus	+
A. tamarii	+
A. ochraceus	+
Aspergillus sp. 125	+
Penicillium candidofulvum, strain A	+
" " " B	+slow*
" " " C	—
P. chrysogenum, strain A	+
" " " B	+

TABLE I—*contd.*

Species	Viability after two years (or 18 months where indicated with asterisk).
P. expansum	—
P. lilacinum	—
P. pinophilum	—
P. luteum	+
P. purpurogenum	+
P. rugulosum	+
Penicillium sp. 209 B	+
Penicillium sp. 138 G	+
Scopulariopsis brevicaulis	+
Cladosporium herbarum, strain A	+*
,, ,, ,, B	+
Trichoderma lignorum	+*
Rhizopus arrhizus	+*
Rhizopus sp. 6	+*
Mucor sp. 19	+*
Mucor sp. 210 B	+*
Trichothecium roseum	—*
Thamnidium sp. 190	+
Stachybotrys ? atra	+
Stemphylium sp. 207 A	+
,, ,, 207 B	+
Chaetomium sp. 206	+
Memnoniella echinata	+

It will be seen that forty-one out of the forty-seven species survived. Of those that did not survive, *Trichothecium* has thin-walled spores, and the remainder comprise four *Penicillium* spp. and an *Aspergillus*. The results are based on one sealed culture of each type.

The second series of experiments comprises a few sand cultures sealed in England in 1933, and two years later brought to India and opened on arrival. Results are shown in Table II.

TABLE II

Sand cultures

Species	Viability after two years
Aspergillus versicolor	+
A. flavus (in duplicate)	+
A. oryzae	—
A. ochraceus	+
A. candidus	+
Scopulariopsis sp. 221	+
Stachybotrys ? atra	—
Chaetomella sp. 238	+

The third series consists of sand cultures sealed in India in 1935 and opened a year later. A number of plant pathogens were included, and the cultures were stored at Indian laboratory temperatures, which reached 35°C. during the hottest months. Results are shown in Table III.

TABLE III

Sand cultures

Species	Viability after one year
Aspergillus nidulans	+
Trichoderma lignorum, strain A	+
,, ,, ,, B	—
Colletotrichum falcatum	—
Sclerotium rolfsii (sclerotia)	+
Sclerotium oryzae (sclerotia)	—
Rhizoctonia microsclerotia (sclerotia)	+
Ustilago hordei	+
U. kolleri	+
U. tritici	—
U. scitaminea	—

tested for spore germination only

Sealed cultures in sugar solution

This is claimed to give good results in some hands, but the exact methods used do not appear to be recorded. The writer's attempts to make use of sugar solutions have been less successful, but two series of experiments are recorded in Tables IV and V.

In the first series, various mould fungi were grown in a nutrient solution (Czapek's) modified to contain ten per cent cane sugar and one per cent chalk. The fungi were allowed to grow until freely sporing and the tubes were then sealed off. These cultures were made in England, taken to India after six months, and opened in India in February 1935 about nine months after the time of sealing. It will be seen from Table IV that two-thirds of the cultures proved non-viable.

TABLE IV

Cane sugar cultures

Species	Viability after nine months
Aspergillus amstelodami	—
A. ruber	—
A. herbariorum minor	+
A. nidulans (non-perithecial)	—
A. niger	+

TABLE IV—*contd.*

Species	Viability after nine months
A. versicolor	+
A. Sydowi	—
A. terreus	+
A. flavipes	+
A. Wentii	—
A. ochraceus	—
A. oryzae	+
A. ustus	—
A. tamarii	—
Penicillium candidofulvum	—
P. pinophilum	—
P. purpurogenum	+
P. rugulosum	—
P. spinulosum	—
Penicillium sp. 239	—
Scopulariopsis brevicaulis	+
Paecilomyces varioti	—
Byssochlamys fulva	+
Mortierella sp. 248	—
Syncephalastrum sp. 192	+
Chaetomella sp. 238	+
Stemphylium sp. 214	—
Stachybotrys ? atra	—
Acrothecium lunatum	—
Stysanus sp. 255	—
Memnoniella echinata	—

A few similar cultures were sealed in India in April 1935 but with further modifications. In the first place, ten per cent dextrose was used in place of ten per cent saccharose, and secondly the transfer made was of a portion of an agar culture containing active mycelium, the tubes being sealed immediately after inoculation. The viability was tested after one year's storage at Indian laboratory temperatures.

TABLE V

Dextrose cultures

Species	Viability after twelve months
Aspergillus nidulans	+
A. niger	—
Trichoderma lignorum, strains A and B	—
Chaetomium indicum	—
Colletotrichum falcatum	—
Phytophthora spp., 4 strains	—

It will be seen from Tables IV and V that both methods of storage in sugar solution gave results inferior to those obtained with sand.

Bacterial cultures

The storage of bacterial cultures does not come in the scope of the present paper, but it may be mentioned for purposes of comparison that the sand culture method is applicable to spore-forming bacteria, the bacterial spores showing even greater viability in this respect than fungal spores.

Non-sporing bacteria, such as the plant pathogenic forms, may be sealed as liquid cultures in broth containing one per cent dextrose with chalk added to maintain a neutral reaction. A culture of *Streptococcus lactis* made in this way was active after 2½ years' storage in England ; of two plant pathogens whose viability was tested after seven months storage in India, a bacterial leaf-streak organism from maize was non-viable, whilst the Red-stripe organism from sugarcane was still active.

Discussion

The results given in this paper indicate that sand cultures afford a useful means of storing certain fungi, in particular those whose spores are thick-walled. It may be anticipated that such spores will retain their viability for a year or two in this state, but sand storage should only be regarded—except for well-tried species—as an additional and not as an alternative means of storing valuable cultures. In interpreting the results shown in Tables I—III, it must be remembered that each record is based on a single culture, and that non-viability may in some cases be due to faulty technique in making this culture, *e.g.*, use of a hot needle, failure to transfer sufficient spores, use of immature cultures, or accidental heating of the sand during the sealing process.

When sand cultures can be relied upon, they present a number of advantages. All chance of infection by mites or moulds is definitely ruled out. The cultures are compact, not easily broken, and seldom require sub-culturing.

A further point—on which few data are yet available—is that sand cultures are more likely to retain their characteristic features than slope cultures. Species such as *Helminthosporium* when kept on agar media for a number of sub-cultures, are apt to show considerable variation or even to produce marked saltants. The presence of toxic substances [Galloway, 1933] and unfavourable physical conditions have been shown to increase the tendency to saltation, and it is reasonable to assume that such conditions are more likely to occur in old vegetative cultures on agar than in dry spores on sand. Additional evidence that saltants are due to staling products is supplied by the fact that they occur more readily in rich media than in media poor in nutrients [Mitra, 1931].

Summary

Results are recorded for the storage of a number of fungi, mainly saprophytic moulds, in sealed culture for one or two years.

Many fungi possessing resistant spores or sclerotia are capable of retaining their vitality for long periods when stored on dry sand in sealed tubes. This affords a method of keeping valuable cultures safe from all chance of infection. Sand cultures alone, however, should not be relied upon.

Two methods of storage in sugar solutions were tested, but gave unsatisfactory results compared with storage on sand.

Acknowledgments

Acknowledgments are made to Dr. A. C. Thaysen, in whose laboratory the writer, as far back as 1921, learnt the use of sand for preserving bacterial spores; and to Dr. R. H. Pickard, Director of the British Cotton Industry Research Association, for permission to make use of the data given in Table I.

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BASE-EXCHANGE STUDIES, I

A PRELIMINARY STUDY OF THE EFFECT OF CERTAIN CATIONS SATURATING* THE SOIL'S EXCHANGE COMPLEX, ON ITS PHYSICOCHEMICAL PROPERTIES, AND THEIR RELATION TO THE PLANT GROWTH

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INTRODUCTION

The authors [Singh and Nijawan, 1932] while working on *bari* soil observed that there is a marked improvement in the physical condition of the soil on treatment with soluble calcium salts. *Bari* soil in its original state is extremely hard, difficult to plough, and entirely lacking in permeability. It contains eighty-five to ninety per cent of monovalent exchangeable bases, and does not support vegetation. But when this soil is treated with soluble calcium salts, such as calcium chloride or gypsum, its physical properties are modified, and it is able to support a crop of wheat. The question, therefore, arises whether an entire absence of plant growth on these soils is merely due to a direct toxic effect of a high replaceable sodium content, as suggested by Kelley and Brown [1924, 1925] or due to unfavourable physical conditions prevailing in the soil. The replacement of sodium by calcium in the exchange complex brings about a change in the physical condition of the soil, but whether this replacement is also accompanied by such chemical and biological changes as liberate certain plant food materials essential for the growth of the plant, has yet to be determined. It is the object of this paper to report the results of a preliminary investigation on the relationship of different exchangeable cations, such as calcium, magnesium, manganese, potassium, ammonium and sodium saturating the soil's exchange complex with such physical properties as : (1) rate of percolation, (2) water-holding capacity, (3) dispersion coefficient, and (4) hydrogen-ion concentration, on the one hand, and their relationship to plant growth and the plant food material removed by the crop on the other.

*The term 'saturation' employed in this paper has a restricted meaning. This merely implies that a soil is saturated with regard to a particular base, when it can take no more of that base in the exchange complex with further leachings under the conditions of the experiments.

Experimental

The soil selected for these experiments was *bari* soil from Montgomery district. It was a medium loam, consisting mainly of sodium clay. The analysis of the soil is given in Table I. It is deficient in nitrogen and organic matter, but possesses all other plant food materials in sufficient quantity. Owing to its being deficient in organic matter, the biochemical changes in this soil are of little significance. Besides, such a soil is highly suitable for the purpose of the study outlined above, as the plant food material which is rendered available as the result of cationic exchange is derived mainly from the inorganic complex.

Table I

(*Per cent on air-dried soils*)

	Per cent
1. Mechanical analysis	
Clay	20·16
Fine silt	15·09
Silt	22·14
Fine sand	41·11
Coarse sand	0·72
Gravel	0·35
2. Chemical analysis	
Insoluble residue	76·85
Soluble silica	0·11
Iron (Fe_2O_3)	4·93
Aluminium (Al_2O_3)	8·54
Calcium (CaO)	3·08
Magnesium (MgO)	1·45
Phosphorus (P_2O_5)	0·25
Potassium (K_2O)	1·16
Sodium (Na_2O)	0·87
Organic matter	0·28
Nitrogen	0·03
3. Available analysis	
Phosphates	0·10
Potassium	0·09
4. Exchangeable bases	
Calcium	0·0307
Magnesium	0·0157
Sodium	0·2160
Potassium	0·0412

Preparation of soils saturated with different cations

The soil after being well powdered was passed through a two-mm. sieve. In order to prepare soils saturated with different cations, one hundred grams of the

soil with 150-200 c.c. of warm normal solutions of the chlorides of calcium, magnesium, manganese, potassium, and ammonium was thoroughly shaken, and kept overnight. Next day the soil was transferred to a Büchenr funnel fitted with a filter paper, and filtered under slight but uniform suction and was further leached with 300-400 c.c. of the corresponding salt solutions. The excess of the salt solution was washed down with distilled water. It was observed that soils saturated with potassium, sodium, and ammonium ions could not be leached effectively with distilled water, as owing to their high dispersion clay particles were carried into the filtrate. Consequently, in these cases leaching was done with fifty per cent alcohol. The soils thus leached were dried, crushed and sieved, and were used for the determination of the following physical properties :—

1. Dispersion coefficient.—The dispersion coefficient being the measure of the state of aggregation of soil particles saturated with different cations, was determined according to Keen and Puri's method [1925]. The results tabulated in Table II indicate that soils saturated with divalent bases are less dispersed and form more resistant aggregates than soils saturated with monovalent bases. Of soils saturated with divalent bases, the magnesium soil gives a higher dispersion coefficient than either the manganese or the calcium soil which have about the same dispersion coefficient. Thus soils saturated with manganese and calcium ions are more flocculated than the magnesium-saturated soil.

TABLE II

Description	Dispersion coefficient	Water-holding capacity (moisture as per cent on air-dried soil)	Rate of percolation (time taken by 100 c.c. of water)			Soil reaction (pH value)
				Hrs.	Mts.	
Calcium soil	31·00	43·67	1.	0	25	8·29
			2.	0	27	
			3.	0	27	
Magnesium soil	41·70	39·94	1.	0	41	8·76
			2.	0	63	
			3.	0	81	
Manganese soil	29·40	42·41	1.	0	9	7·36
			2.	0	9	
			3.	0	11	
Ammonium soil	98·00	34·40	1.	1	29	8·62
			2.	2	58	
			3.	3	24	
Potassium soil	99·40	34·50	1.	2	5	9·11
			2.	4	3	
Sodium soil	94·64	29·10	3.	4	7	
			1.	27	32	10·01

2. Water-holding capacity.—The water-holding capacity affords a means of comparing soils on the basis of their capacity to hold moisture, which depends on the pore-space, and colloidal matter present in the soil. Water-holding capacity was determined by Hilgard's method as described by Hall [1921] with modifications of Keen and Raczkowski [1921]. The data in Table II is a mean figure of five determinations made on individual samples, and the variation between the different determinations made on the same soil was not more than 0·5 per cent.

A comparison of the results contained in the above table shows that monovalent ions (K, NH_4 and Na) cause a change in the arrangement of the colloidal material. The soils saturated with divalent bases can hold more moisture than those saturated with monovalent bases. The calcium-saturated soil has the highest water-holding capacity, while the soil saturated with sodium ions has the least. The water-holding capacity goes on decreasing in the order shown below :—

$$Ca > Mn > Mg > K > NH_4 > Na.$$

WATER-HOLDING CAPACITY OF SOIL CONTAINING DIFFERENT AMOUNTS OF EXCHANGEABLE CALCIUM

The results contained in Table III show that the water-holding capacity of a soil increases with an increasing amount of exchangeable calcium in it. The soil having 0·0432 per cent of exchangeable calcium holds 28·91 per cent of moisture, while the same soil containing 0·1824 per cent of exchangeable calcium holds 44·26 per cent of moisture.

TABLE III

Serial No.	Exchangeable calcium as Ca (percentage on air-dried soil)	Maximum water-holding capacity (moisture as percentage on oven-dried soil)	Rate of percolation (time required for 100 c.c. of water to pass through)	
			Hrs.	Mts.
1	0·0432	28·91	27	55
2	0·0624	31·88	27	32
3	0·0720	32·44	25	36
4	0·0992	33·11	24	12
5	0·1008	34·02	24	3
6	0·1104	34·90	7	41
7	0·1120	34·88	4	5
8	0·1184	35·05	2	39
9	0·1304	35·53	2	19
10	0·1334	36·26	1	54
11	0·1352	38·47	1	5
12	0·1824	44·26	0	26

3. Rate of percolation.—The rate of percolation in these soils was determined by the method described by Bouyoucos [1930], with a slight modification. As the pressure of the tap water is variable, a manometer was introduced to measure the pressure, and in all soils water was allowed to percolate under a definite suction force (33 cm.). The method, though not absolute, is yet capable of giving fairly good comparative results which can be repeated under similar conditions.

Fifty grams of the soil were well shaken with fifty c.c. of distilled water, and then placed on the Büchner funnel to which No. 40 Whatmann's filter paper was fitted. The soil adhering to the sides of the beaker was washed into the funnel with another fifty c.c. of distilled water, and the suction started. In order that the soil system may become settled, and more or less in equilibrium, the suction was continued for about fifteen minutes before measurements were commenced on the rate of flow of water. Meanwhile, the entire amount of water was never allowed to filter through, as a re-arrangement of soil particles causes a change in the rate of percolation.

The results contained in Table II show that the different cations in the exchange complex produce a striking effect on the rate of percolation of water. The order of rate of percolation is $Mn > Ca > Mg > NH_4 > K > Na$.

The time taken for 100 c.c. of water to pass through a layer of fifty grams of soil varied considerably in the case of soil saturated with different cations. For instance it took nine to eleven minutes for 100 c.c. of water to pass through a manganese-saturated soil, twenty-five to twenty-seven minutes through a calcium-saturated soil, forty-one to eighty minutes through a magnesium-saturated soil, 89 to 204 minutes through ammonium-saturated soil and twenty-seven hours and thirty-two minutes to pass through a sodium-saturated soil. Further, it will be observed, that the rate of percolation decreases if repeated percolations are obtained through the same column of the soil without disturbing it. The change is more marked in the case of a magnesium-saturated soil than in the case of manganese or calcium-saturated soils (where there is practically no change), and is still greater in the case of ammonium and potassium saturated soils. This is due to the fact that when water remains in contact with soils saturated with different cations, the magnesium, potassium and ammonium soils become more puddled than soils saturated with manganese and calcium ions, and it is on account of this puddling effect produced in these soils that there is a marked decrease in the rate of subsequent percolations.

Rate of percolation in soils containing different amounts of exchangeable calcium

The rate of percolation was studied in twelve soil samples containing different amounts of exchangeable calcium. From the results given in Table III it will be observed that the rate of percolation increases with increasing amounts of exchangeable calcium, but there is a sudden rise in the rate of percolation in the case

of samples containing 0·1104 per cent of calcium. One hundred c.c. of water take twenty-four hours and three minutes to pass through the soil containing 0·1008 per cent of exhangeable calcium, while the same amount of water takes only seven hours and forty-one minutes to pass through the soil containing 0·1104 per cent of exchangeable calcium. In other words, a slight increase in the amount of exchangeable calcium at that particular stage can bring about a great difference in the rate of percolation. This observation is of great practical importance, as this difference in the rate of percolation is due to complete flocculation of the soil which occurs when it contains 0·1104 per cent of exchangeable calcium. Further, a slight increase over 0·1104 per cent in the amount of exchangeable calcium is responsible for a great increase in the rate of percolation. When the soil becomes saturated with exchangeable calcium, 100 c.c. of water take only twenty-six minutes to percolate through it.

4. pH value.—The pH values were determined by the hydrogen electrode in soil suspensions containing 1 : 2 ratio of soil to water.

The results given in Table II clearly indicate that the nature of the exchangeable cations in the exchange complex greatly affects the pH values of the soil. Thus a soil saturated with sodium ions has a pH value of 10·01, but when sodium is replaced by potassium, magnesium, ammonium, calcium and manganese ions, the pH values are altered to 9·11, 8·76, 8·62, 8·29 and 7·36 respectively.

Crop

Five hundred grams of each of the soils saturated with different cations were placed in glass tumblers, and wheat was sown in them. Altogether there were four repeats of each treatment. It was observed, that the seeds in soils saturated with divalent bases germinated earlier than those in soils saturated with monovalent bases. In the case of sodium-saturated soils only a few seedlings made appearance, which died soon after. Next, the plants in the ammonium-saturated soil began to show signs of exhaustion, the leaves began to turn yellow at the tips and soon after all the plants died. In all the remaining tumblers the plants were healthy and strong, except in the case of those soils saturated with manganese, where the plants were small in size with thin stems and narrow leaves. The following is a statement of the yields of the wheat crop in grams per pot :—

	Soil					
	Ca	Mg	Mn	K	NH_4	Na
Total yield . . .	6·85	6·10	6·00	3·5	*nil*	*nil*
Grain	2·10	1·70	0·78	0·1	*nil*	*nil*
Straw	4·75	4·40	5·22	3·4	*nil*	*nil*

Exchangeable bases

Exchangeable bases were determined in soils saturated with different cations according to Hissink's method [1923] with the modification suggested by Kelley and Chapman [1930]. It was observed that it was not possible to replace the bases completely, especially in the case of manganese, by leaching the soil with 500 c.c. of normal ammonium chloride solution. Therefore, instead of leaching the soil with 500 c.c. of the solution, it was leached with 1,000 c.c. to bring about complete replacement, and the leachings were collected separately in two litre flasks.

From the data of the exchangeable bases given in Table IV it will be observed that they could not all be completely replaced, and ratios of cations saturating the exchange complex to the total bases varied considerably.

TABLE IV

Exchangeable bases : Percentage on air-dried soil

Serial No.	Description of the soil	Before sowing crop						After removal of crop					
		Calcium as Ca	Magnesium as Mg	Sodium as Na	Manganese as Mn	Potassium as K	Ammonium as NH_4	Calcium as Ca	Magnesium as Mg	Sodium as Na	Manganese as Mn	Potassium as K	Ammonium as NH_4
1	Calcium soil . .	0·1824	0·0306	0·0144	...	0·0343	...	0·1504	0·0159	0·0374	...	0·0240	...
2	Magnesium soil .	0·0480	0·1204	0·0087	...	0·0438	...	0·0448	0·0928	0·0275	...	0·0927	...
3	Manganese soil. .	0·0656	*Nil*	0·0173	0·2377	0·0240	...	0·1664	0·0115	0·0550	0·2089	0·0515	...
4	Potassium soil .	0·0592	0·0169	0·0181	...	0·3633	...	0·0384	0·0273	0·0550	...	0·3384	...
5	Ammonium soil .	0·1136	0·0076	0·0371	...	0·0275	0·1170	0·0976	0·0011	0·0245	...	0·0412	0·0648
6	Sodium soil . .	0·0303	0·0186	0·2347	...	0·0326	...	...	...	...	...	...	...

In order to ascertain whether it was possible to bring about a greater replacement of all the bases of the soil by prolonged leaching, the soil in question was leached with 5,000 c.c. of hot normal calcium chloride solution. The amount of exchangeable calcium estimated showed that the amount of calcium absorbed was the same whether the soil was leached with three to four hundred c.c., or five thousand c.c., of the solution. These results are in accordance with the observations made by Gedroiz [1931], Kelley and Brown [1927], and Csiky [1932] who assert that complete replacement cannot take place though prolonged leaching as observed by Mattson [1931] brings considerable change in the exchange complex.

The data of exchangeable bases calculated in gram equivalents are given in Table V. From these it will be observed that sodium in the original *bari* soil is replaced by other bases in almost equivalent amounts. Therefore, it follows, that so far as the replacement of sodium by other bases is concerned, this is governed by the ordinary laws of chemical reaction. The only exception is in the case of an ammonium soil where besides a true chemical reaction probably absorption compounds are also being formed.

TABLE V

Exchangeable bases in gram equivalents (Per cent on air-dried soil), before sowing crop

Serial No.	Description	Calcium as Ca	Magnesium as Mg	Potassium as K	Sodium as Na	Manganese as Mn	Ammonium as NH_4	Total bases
1	Calcium soil .	0·0911	0·0252	0·0088	0·0062	..	..	0·1313
2	Magnesium soil .	0·0240	0·1004	0·0112	0·0038	..	..	0·1394
3	Manganese soil .	0·0328	*Nil*	0·0061	0·0075	0·0870	..	0·1334
4	Potassium soil .	0·0296	0·0139	0·0929	0·0079	..	..	0·1443
5	Ammonium soil .	0·0567	0·0063	0·0070	0·0161	..	0·0650	0·1511
6	Sodium soil .	0·0251	0·0133	0·0083	0·1020	..	..	0·1507

MINERAL ANALYSIS OF CROP

When the amounts of various minerals (Table VI) removed by crops from soils saturated with different cations are compared, the following conclusions are arrived at :—

(*a*) Plants remove in excess only that particular base with which the soil is saturated. For instance potassium is removed in excess from a potassium-saturated soil, magnesium from magnesium-saturated soil and so on.

(*b*) The figures of ash percentages show that the amount of mineral matter removed by crops is greatly influenced by the nature of the cations on the exchange complex. The total mineral contents removed by crops from soils saturated with manganese, magnesium, potassium and calcium are 10·04 per cent, 15·74 per cent, 20·04 per cent and 17·05 per cent, respectively.

(*c*) Perkin [1932], while working on the availability of phosphorus in soils saturated with different cations, observed that more soluble phosphates are formed in the case of soils saturated with iron, aluminium, and magnesium, than in the case of soils saturated with hydrogen, calcium, ammonium, and potassium ions, in which the solubility of phosphates is decreased. The amount of phosphoric acid removed by the crop as shown in Table VI, also confirms this observation of Perkin that the phosphate content of crops from a magnesium-saturated soil is greater than the phosphate content of crops from a soil saturated with other cations.

(*d*) The amount of monovalent bases, namely sodium and potassium removed by a crop from soils saturated with different cations increases with an increase in their pH values. The total amount of potassium and sodium removed by crops from a manganese-saturated soil having a pH value of 7·36 is 2·77 per cent, from a calcium-saturated soil with a pH value of 8·29 is 4·72 per cent, from a magnesium-saturated soil with a pH value of 8·76 is 6·25, and in the case of a crop from a potassium-saturated soil with a pH value of 9·11 the amount of these bases is 10·20 per cent.

TABLE VI

Analysis of wheat crop (per cent on air-dried crop)

Serial No.	Description	Ash	Insoluble residue	Iron and aluminium as Fe_2O_3 and Al_2O_3	Calcium as CaO	Magnesium as MgO	Manganese as Mn_3O_4	Sodium as Na_2O	Potassium as K_2O	Phosphorus as P_2O_5
1	Calcium-saturated soil.	17·05	5·820	1·2176	1·4240	0·6971	0·0100	0·8547	3·870	0·5368
2	Magnesium-saturated soil.	15·74	4·160	0·7351	0·7204	1·1920	0·0100	1·3880	4·869	0·6248
3	Manganese-saturated soil.	10·04	2·634	0·5790	0·7456	0·5217	0·0200	0·2876	2·493	0·5637
4	Potassium-saturated soil.	20·40	4·471	0·6388	0·5905	0·5297	0·0120	0·7958	9·414	0·5412

In Table VII all the minerals are expressed as percentages on ash, and for the sake of comparison the average figures of analyses of the mineral contents of a wheat crop from a normal soil depicted as 'percentage on ash' are also given. From these results it will be observed that calcium is not being removed by the crop from the calcium-saturated soil to the same extent as magnesium, manganese and potassium from the corresponding saturated soils. Large amounts of manganese, magnesium and potassium in the soil's exchange-complex induce an excessive absorption of these bases by the crops.

TABLE VII

Mineral contents on 100 grams of ash

Serial No.	Description	Insoluble residue	Iron and aluminium as Fe_2O_3 and Al_2O_3	Manganese as Mn_3O_4	Calcium as CaO	Magnesium as MgO	Phosphorus as P_2O_5	Sodium as Na_2O	Potassium as K_2O
1	Calcium soil .	34·14	7·14	5·85	8·35	4·09	3·25	5·01	22·70
2	Magnesium soil.	26·44	4·67	4·20	4·58	7·57	3·97	8·82	30·94
3	Manganese soil.	26·23	5·77	12·10	7·43	5·20	5·65	2·86	24·83
4	Potassium soil .	22·30	3·18	3·82	2·94	2·64	2·70	3·97	46·97
5	Normal soil .	50·63	5·24	0·58	7·41	4·13	6·86	1·96	24·03

Discussion

It is evident from the results obtained above that the nature and quantity of cations on the exchange complex are responsible for a change in the structure of the soil. The cations are not only responsible for a change in the physical properties of the soil, but they also govern the physico-chemical and chemical processes occurring in it. Therefore, if the introduction of certain cations in the soil complex improves the physical properties of the soil, it does not necessarily follow that it also increases the crop-producing capacity of the soil. For example, the introduction of an ammonium ion in the exchange complex improves the permeability and water-holding capacity of the soil, but the soil thus treated is unable to support the growth of a crop. On the contrary, a potassium soil with a higher pH value and a slower rate of percolation than an ammonium soil shows, is able to support a good crop. Again, a manganese soil is more permeable and has the same water-holding capacity as a calcium soil, but the plants which germinated in it were poor, with weak stems and narrow leaves. The data for ash percentages also show that plants growing in a manganese soil remove the smallest quantity of minerals.

Stability of calcium in the exchange complex

(*a*) It has been observed that when plants are grown on soils saturated with various cations, there is excessive absorption of these bases by the crop. However, calcium from a calcium-saturated soil is not removed to the same extent as other cations, like potassium, magnesium and manganese. This observation brings out the fact that various cations are held up in the soil complex in different ways and that calcium cations are in a more stable combination with the exchange complex than are other cations.

(*b*) The analyses of a manganese-saturated soil (Table IV) show that the amount of exchangeable calcium in the soil increases from 0·0656 to 0·1664 after the removal of the crop. This clearly shows that manganese in a manganese-saturated soil is not sufficiently strongly held in the exchange complex and is being constantly replaced by calcium.

(*c*) Similarly in the case of potassium and magnesium saturated soils, there is a considerable increase in the exchangeable sodium, and decrease in the exchangeable calcium in the soil after the removal of the crop. This shows that in these soils sodium is constantly replacing potassium and magnesium in the exchange complex, or in other words, magnesium and potassium are comparatively loosely held in the exchange complex. Consequently any improvement brought about by these cations will involve only a temporary improvement.

(*d*) With the exception of a potassium-saturated soil, a calcium-saturated soil removes the greatest quantity of minerals. In this connection it has already been pointed out that a high ash percentage is due to an excessive uptake of potassium by the crop which forms more than fifty per cent of the total mineral matter. This fact further shows the superior quality of the crop grown on a calcium-saturated soil.

From the foregoing discussion it follows that the various cations not only affect the physical properties of the soil, but also profoundly modify its chemical properties. Further, an improvement in the physical properties does not necessarily follow an improvement in the chemical properties of the soil. Finally it appears that a calcium soil is the most stable and best suited for plant growth.

Summary and conclusions

1. A preliminary study of the effect of various cations saturating the soil's exchange complex on the physical and physico-chemical properties of the soil is presented.

2. From the results obtained it appears that the physical properties of a soil such as the dispersion coefficient, the water-holding capacity, the rate of percolation and the pH values are greatly influenced by the nature and amount of cations in the exchange complex.

3. A study of the rate of percolation and water-holding capacity of a soil containing different amounts of exchangeable calcium is invariably followed by an increase in the rate of percolation and water-holding capacity of the soil.

4. A definite amount of exchangeable calcium is required to bring about complete flocculation of the soil, and when this amount is reached, there is a sudden increase in the rate of percolation. Further, a slight increase in the amount of exchangeable calcium beyond that point brings about a correspondingly greater increase in the rate of percolation.

5. Sodium and ammonium saturated soils do not support a crop, but soils saturated with calcium, manganese, magnesium and potassium can do so.

6. There is a certain relationship between the plant food material removed by a crop and the nature of the cations saturating the soil's exchange complex.

(*a*) When a particular base is in excess in a soil it is found that crops tend also to remove that base in excess. The amount of such bases removed varies with the nature of the cations. Manganese, magnesium and potassium are removed in double the quantity, and calcium in almost the same amount as is removed by the crop from a normal soil.

(*b*) The availability of phosphorus is increased by the presence of magnesium, and decreased by the presence of calcium and potassium in the soil's exchange complex.

(*c*) The total amount of minerals present in the crop varies with the nature of the cations, and follows the order given below :—

$$K > Ca > Mg > Mn.$$

7. The pH value of the soil is greatly affected by the nature of the cations.

8. The amount of monovalent bases removed by the plant increases with an increase in the pH value.

9. It does not necessarily follow that an improvement in the physical properties of the soil makes it fit for plant growth, as the nature of the cations also in-

fluences the chemical properties of the soil. Soils saturated with calcium ions are more stable and bring about such physical and chemical changes in the soil as are favourable to the growth of a good crop.

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SOME STUDIES ON THE SCLEROTIAL DISEASES OF RICE (*SCLEROTIUM ORYZAE* CATT.) IN THE PUNJAB

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(With Plate LIV)

CONTENTS

	PAGE
1. INTRODUCTORY	973
2. SYMPTOMS OF THE DISEASE	974
3. DISTRIBUTION OF THE DISEASE	975
4. INFECTION EXPERIMENTS—	
I. Material and methods	976
II. Pot experiments	976
III. Field experiments	979
5. METHODS OF PERPETUATION OF THE DISEASE	982
6. SUMMARY	984
7. ACKNOWLEDGMENTS	984
REFERENCES	984

1. Introductory

A disease of rice was reported in 1932 from the Rice Farm, Kala Shah Kaku, causing a considerable damage to the rice crop in certain fields. The disease, in addition to causing a marked decline in yield, interfered with the breeding work of the station as many plants died altogether as a result of it in the experimental plots. On examination of the affected plants on the spot the disease was identified as caused by *Sclerotium orzyae* Catt. In the same season specimens of diseased rice plants showing rot of the stem and infected with *Sclerotium orzyae* were also received from Gujranwala, Gurdaspur, Sialkot, Sheikhupura and Kangra (Palampur) districts.

The loss in yield caused by the disease in the affected fields at the Rice Farm, Kala Shah Kaku, varied from five to eighty per cent. In other places the average loss caused by the disease was reported by the local Agricultural

Assistants to vary from five to twenty-five per cent while many fields were free from the disease. From these figures it is not possible to estimate the total amount of loss caused by the disease in the Province but it is apparent that this malady ranks as one of the major diseases of farm crops in the Punjab. The study of the disease was therefore taken in hand. The present paper deals with the symptoms, distribution and perpetuation of the disease in the Punjab and pathogenicity of the causal fungus studied in pot culture and field experiments. In a later publication, it is intended to give factors affecting the incidence and development of the disease and methods of controlling it.

2. Symptoms of the diseases

The most important and general symptom of the disease as observed in the Punjab is Stem-Rot. But considerable variation has been observed with regard to the extent of Stem-Rot and the time of its occurrence.

I. Stem-rot

When the plants are about two to three months old, *i.e.*, about the beginning of September, the leaf-sheaths and leaves at the first, second or third internode from the base of the plant and up to the part where irrigation water reaches, begin to wither and ultimately dry up. The sheaths soon turn somewhat dark and begin to rot. At this time sclerotia of the fungus appear as small black dots at the bases of dried leaves and within the leaf-sheaths. Then the stem below the diseased sheaths gets infected. Generally the node from where the diseased leaves arise is first attacked. At first a small darkish spot appears and then infection spreads upward and in some cases downward also, and involves the whole internode below the affected leaf-sheath. The infected part of the stem then begins to rot and becomes so soft that it collapses and the plant falls down. In severe cases, it occurs either before the emergence of ears or when the ears have just come out. In such cases, the affected shoot dies altogether or if it remains green, the ears remain totally or partially sterile. If the Stem-Rot appears a little later, grains may be formed, but they are very much shrivelled. If the attack is very late, normal grains may be produced. Thus the extent of the damage depends upon the time when the Stem-Rot sets in (Plate LIV). In all these cases sclerotia of the fungus appear in large numbers inside the affected stem.

II. Drying of basal leaves

In some cases leaf-blades and leaf-sheaths dry on account of the disease but the stem does not rot. In such cases almost normal grains are produced and no appreciable damage to the grains is caused. The sclerotia of the fungus are, however, produced on dried leaf-sheaths.

(*a*) Showing rot of the first internode of a rice plant.
(*b*) ,, ,, ,, second internode of a rice plant.
(*c*) ,, ,, ,, third internode of a rice plant.
(*d*) Showing Sclerotia of '*Sclerotium oryzae*' on the base of a leaf sheath.
(*e*) Showing Sclerotia of '*Sclerotium oryzae*' inside the affected stem of a rice plant.

III. DISCOLOURATION OF BASES OF STEMS

A large number of cases have also been observed where neither the leaves are attacked nor Stem-Rot appears, but the bases of stems of plants, all the same, show discolouration and bear sclerotia. In such cases the plant generally does not collapse. The affected plant either wilts or if it remains green it produces ears which remain sterile or contain shrivelled grains. If, however, the attack is late normal grains are produced.

IV. PRESENCE OF SCLEROTIA ON STUBBLE

Many cases have also been observed when no symptoms of the disease were noticeable in the standing crop, but after harvest sclerotia of the fungus were found in abundance on stubble.

Excessive and late tillering as described by Butler [1913] and Shaw [1913], whose observations were confined to Bengal, Bihar and Burma, have not been noticed so far in the Punjab, though as above stated, sterility and discolouration of the bases of plants as described by them is fairly common in the Punjab. The Stem-Rot which is the most important symptom of the disease in the Punjab has not been recorded by them. Mundkur [1935], the latest worker on this disease at Pusa (Bihar) whose study is limited chiefly to Pusa and its surroundings and who also visited Anakapalle, Samalkota, Maruteru, Coimbatore and Pattambi in the Madras Presidency and Karnal in the Punjab, did not observe any of the symptoms of the disease though he found sclerotia of the fungus in many plants. It is, however, interesting to note that as in the Punjab, stem-rot is the most important symptom of the disease in many countries abroad as described by Cattaneo [1879] and Briosi and Cavara [1886] in Italy, Reyes [1929] in Philippines, Vincens [1923] in Indo-China, Tisdale [1921] in Louisiana (U. S. A.), and Tullis and Cralley [1933] in Arkansas, Texas and Louisiana (U. S. A.). The nature of damage caused by the disease in these countries is also identical with the Punjab. Park and Bertus [1932] state that in Ceylon the disease causes death of seedlings, and the advanced plants when affected produce abnormal tillers, and the stalk gradually turns yellow and dies.

3. Distribution of the disease

During the last three years, specimens of diseased rice plants affected with *Sclerotium oryzae* and showing the Stem-Rot symptom have been received from Sheikhupura, Gujranwala, Gurdaspur, Sialkot, Palampur and D. G. Khan districts. In 1935, after Mundkur [1935] published his paper on this disease and stated that he did not notice Stem-Rot symptom of the disease in Bihar, important rice-growing tracts in the plains of the Punjab were visited to make further observations on the disease. The disease exhibiting acute Stem-Rot was found fairly common in Sheikhupura, Gujranwala and Karnal districts, but it was not observed in Hoshiarpur and Muzaffargarh districts. The other places were not visited.

It is interesting to remark here that Mundkur visited Karnal in the Punjab but did not find the Stem-Rot symptom of the disease though he found sclerotia of the fungus in leaf-sheaths of plants which had well filled up panicles. This seems to be due to the fact that in Karnal district, the disease is serious in certain parts only, *e.g.*, south-eastern villages near Karnal along the bank of the Western Jamna Canal where in 1935 it caused about thirty per cent damage in certain fields, while in other parts of the district the disease is either absent or is found in traces only. Mundkur probably happened to visit those places in the Karnal district where the disease exhibiting Stem-Rot symptom was absent.

4. Infection experiments

I. Material and methods

In the Punjab, rice is grown by sowing the seed directly and also by transplanting seedlings. For infection experiments, rice was grown by both these methods. Nineteen varieties of rice were included in these experiments. The seed of all the varieties was obtained from the Assistant Cerealist, Rice Farm, Kala Shah Kaku, District Sheikhupura, Punjab. Before sowing, the seed was disinfected in 0·1 per cent mercuric chloride solution for fifteen minutes. The infection experiments were conducted both in pots and field.

For infecting the soil, the infected rice stubble and pure culture of the fungus were mixed thoroughly in the upper three-inch layer of soil. The infected rice stubble was obtained from the Rice Farm, Kala Shah Kaku, and before mixing it with the soil, it was chopped up into fine pieces. The culture of the fungus used was grown on oatmeal agar (Oatmeal 50 grms. and agar-agar 20 grms. made up to one litre in distilled water) at 30°C. The culture used was always fifteen to twenty days old. The direct inoculation of rice plants was done by placing a bit of mycelium along with sclerotia of the fungus on the part of the plant to be inoculated and then tying cotton wool over it which was kept moist for fourteen days. Both pot and field experiments were conducted at Lyallpur and canal water was used for irrigation.

II. Pot experiments

(*i*) *By sowing the seed directly in pots*

Forty flower pots filled with clay soil were sterilized in an autoclave at 120°C. for one hour. The soil of ten pots was infected with diseased rice stubble and of ten more with pure culture of the fungus on 20th May 1933. The remaining twenty pots were left as control. Disinfected rice seed of Punjab variety No. 349 was sown, six seeds in each pot, on 1st June 1933. All the pots were watered thrice daily till the crop matured. The plants were kept under constant observation throughout this period. The results are given in Table I.

The experiment could not be arranged in 1934 but was carried out in 1935, when the soil was infected on 1st June and the seed was sown on 5th June. These results are also given in Table I.

TABLE I

Results of infection experiments in pots by sowing the seed

Year	Treatment	Total No. of plants	No. of diseased plants			
			Showing Stem-Rot symptoms	Showing drying of leaves at the bases of plants	Total	Per cent infection
1933	Soil infected with diseased rice stubble.	57	2	3	5	8·8
	Soil infected with pure culture of the fungus.	56	3	5	8	14·3
	Soil uninfected (control) .	112	0	0	0	0
1935	Soil infected with diseased rice stubble.	52	4	4	8	15·4
	Soil infected with pure culture of the fungus.	51	2	3	5	9·8
	Soil uninfected (control) .	108	0	0	0	0

(*ii*) *By transplanting*

(*a*) *By raising the seedlings in uninfected soil.*—This experiment was carried out in the same manner as (*i*) above, with the only difference that, instead of seed, forty days old seedlings raised in sterilized pots were transplanted on 10th July 1933 in forty pots (ten infected with diseased rice stubble, ten infected with culture of the fungus and twenty uninfected).

The experiment was carried out in 1934 and 1935 also. Thirty-five days old seedlings were transplanted on 10th July 1934, and thirty-seven days old seedlings were transplanted on 12th July 1935. The results of experiments of 1933, 1934 and 1935 are given in Table II.

(*b*) *By raising seedlings in infected soil.*—This experiment was conducted as (*ii*) (*a*) above, with the difference that seedlings were raised in pots the soil of which was infected with pure culture of the fungus just before sowing the seed.

The experiment was carried out in 1933 and 1934 and the results are given in Table III.

TABLE II

Results of infection experiments by transplanting (seedlings raised in uninfected soil)

Year	Treatment	Total No. of plants	No. of infected plants			
			Showing Stem-Rot symptoms	Showing only drying of leaves at the bases of plants	Total	Per cent infection
1933	Soil infected with diseased rice stubble.	55	3	6	9	16·4
	Soil infected with pure culture of the fungus.	53	4	2	6	11·3
	Soil uninfected (control) .	115	0	0	0	0
1934	Soil infected with diseased rice stubble.	54	4	4	8	14·8
	Soil infected with pure culture of the fungus.	56	3	4	7	12·5
	Soil uninfected (control) .	114	0	0	0	0
1935	Soil infected with pure culture of the fungus.	58	2	19	21	36·2
	Soil uninfected (control) .	60	0	0	0	0

TABLE III

Results of infection experiments by transplanting (seedlings raised in infected soil)

Year	Treatment	Total No. of plants	No. of diseased plants			
			Showing Stem-Rot symptoms	Showing only drying of leaves of plants	Total	Per cent infection
1933	Soil infected with diseased rice stubble.	52	3	4	7	13·5
	Soil infected with pure culture of the fungus.	56	2	2	4	7·1
	Soil uninfected (control) .	57	0	2	2	3·5
1934	Soil infected with diseased rice stubble.	53	4	5	9	17·0
	Soil infected with pure culture of the fungus.	54	4	4	8	14·8
	Soil uninfected (control) .	53	1	1	2	3·8

(iii) By direct inoculation

The seedlings of rice raised in uninfected soil were transplanted in pots containing healthy soil on 12th July 1935. The stems of these plants were inoculated with pure culture of the fungus at the base, and also at heights varying from half an inch to two inches above the base on 7th September 1935 and 21st September 1935. Observations were recorded on 20th October 1935 and the results are given in Table IV.

TABLE IV

Result of direct inoculation experiments

Date of inoculation	Part inoculated	Total No. of plants	No. of diseased plants			
			Showing Stem-Rot	Showing drying or rotting of leaves	Total	Per cent infection
7th Sept. 1935.	Soil infected . . .	58	2	19	21	36
	Bases of stem. . .	24	4	4	8	33
	Stem 1½ in. above the base.	25	1	4	5	20
	Control	20	0	0	0	0
21st Sept. 1935.	Soil infected . . .	23	1	3	4	17
	Bases of stem . .	25	5	4	9	36
	Stem one in. above the base	25	2	15	17	68
	Stem two in. above the base.	25	8	7	15	60
	Control	24	0	3	3	12

Results

In all the above pot experiments the results of which are given in Tables I to IV the fungus was isolated from diseased plants and was always found to be *Sclerotium oryzae* and the diseased plants showed characteristic Stem-Rot symptoms or the drying of basal leaves. These experiments therefore prove that the fungus *Sclerotium oryzae* is pathogenic on rice plants and causes Stem-Rot or drying of basal leaves.

III. FIELD EXPERIMENTS

(i) By sowing the seed directly in the field

A piece of land was divided into four plots, each measuring 30 ft. × 20 ft. The soil of the first and third plots was infected on 15th January 1933 with diseased rice stubble and pure culture of the fungus respectively. Disinfected seed of nineteen varieties was sown on 1st June 1933 in every plot. In each plot there were two sets of nineteen lines each and in each set every variety was sown in one line each.

The experiment was repeated in 1934, when again soil of the first and third plots was infected with diseased stubble and pure culture of the fungus on 15th May. The seed was sown on 5th June. The results of experiments of both the years are given in Table V.

(ii) By transplanting

(a) By raising the seedlings in uninfected soil.—This experiment was conducted in the manner as III *(i)* above with the difference that six-weeks old seedlings of nineteen varieties raised in another piece of uninfected land were transplanted in the four plots on 10th July 1933. The experiment was repeated in 1934 when the seedlings of the same age were transplanted on 15th July.

In 1935 the experiment was conducted again. One portion of a healthy plot was infected with pure culture of the fungus on 1st July and the other was left as control. Five-weeks old seedlings of nineteen varieties were transplanted in these plots on 15th July. The results of all the three years' experiments are given in Table VI.

(b) By raising the seedlings in infected soil.—The plan for this experiment was the same as for *(ii)* *(a)* above with the difference that the seedlings for transplanting were grown in soil which was infected with pure culture of the fungus. The experiment was carried out for two years, 1933 and 1934. The results are given in Table VII.

TABLE V

Results of infection experiments in the field by sowing seed

Serial No.	Variety	Percentage infection							
		1933				1934			
		Diseased stubble added	Healthy soil	Culture of fungus added	Healthy soil	Diseased stubble added	Healthy soil	Culture of fungus added	Healthy soil
1	14	27·5	0	11·7	0	9·1	0	54·6	1·8
2	225	0	0	12·6	0	9·1	0	0	0
3	46	0	0	0	0	4·3	0	31·0	0
4	349	5·0	0	1·7	0	4·1	0·5	48·3	0
5	346	0	0	0	0	6·7	0	9·4	0
6	22	0	0	3·0	0	0	0	0	0
7	7	0	0	4·7	0	0	0	0	0
8	278	4·0	0	5·1	0	3·4	0	18·3	0
9	K. V. 22/28	0	0	0	0	7·9	0	0	0
10	K. V. 38A/28	0	0	0	0	26·2	0	0	0
11	360, 370, 3/30, 337A, 41, 202, 62, 12(S) K. V. 21/28.	0	0	0	0	0	0	0	0

TABLE VI

Results of infection experiments in field by transplanting seedlings growing in uninfected soil

Serial No.	Variety	Percentage infection									
		1933				1934				1935	
		Diseased stubble added	Healthy soil	Culture added	Healthy soil	Diseased stubble added	Healthy soil	Culture added	Healthy soil	Culture added	Healthy soil
1	14	5·0	0	0	0	41·7	2·1	80·1	8·0	29·2	0
2	225	17·5	1·0	6·2	0	9·1	0	31·0	0	43·1	0
3	46	0	0	0	0	4·3	0	39·9	0	11·7	0
4	349	11·4	0	5·7	0	3·5	0	48·3	4·5	19·4	0
5	346	0	0	0	0	6·5	0	10·1	0	0	0
6	360	0	0	0	0	0	0	0	0	25·0	0
7	22	7·1	0	0	0	0	0	0	0	35·5	0
8	3/30	0	0	0	0	0	0	0	0	2·1	0
9	337A	7·5	0	0	0	0	0	0	0	5·5	0
10	62	0	0	0	0	0	0	0	0	6·7	0
11	12(S)	0	0	0	0	0	0	0	0	33·1	0
12	278	13·1	0	0	0	0	0	23·5	3·5	26·1	0
13	K. V. 22/28	25·0	0	0	0	7·9	0	37·0	0	15·0	0
14	K. V. 21/28	0	0	0	0	0	0	0	0	9·7	0
15	K. V. 38A/28	33·3	0	0	0	4·0	0	0	0	16·3	0
16	370, 41, 7, 202	0	0	0	0	0	0	0	0	0	0

TABLE VII

Results of infection experiments in field by transplanting seedlings grown in infected soil

Serial No.	Variety	Percentage infection 1933				Percentage infection 1934			
		Diseased stubble added	Healthy soil	Culture added	Healthy soil	Diseased stubble added	Healthy soil	Culture added	Healthy soil
1	14	35·9	16·1	33·8	0	30·0	0	10·3	0
2	225	15·4	18·2	36·8	1·0	36·2	0	55·7	2·0
3	46	17·3	8·1	20·0	0	28·0	0	20·0	0
4	349	21·8	12·5	33·9	0	40·9	0	43·7	0
5	346	0	0	0	0	17·2	0	3·7	0
6	360	2·9	0	0	0	8·0	0	0	0
7	22	2·4	0	6·2	0	11·1	0	25·1	0
8	370	0	0	1·5	0	10·2	0	0	0
9	337 A	0	0	0	0	3·4	0	3·5	0
10	41	1·2	0	0	0	3·5	0	3·5	0
11	12 (S)	1·2	0	0	0	0	0	5·5	0
12	278	16·4	13·8	40·0	0	75·4	14·9	37·5	0
13	K. V. 22/28	7·5	18·1	25·0	0	30·0	0	54·0	0
14	K. V. 21/28	1·5	0	2·6	0	0	0	22·2	0
15	K. V. 38A/28	39·9	1·0	27·2	0	36·2	0	55·7	0
16	3/30, 7, 202, 62	0	0	0	0	0	0	0	0

Results

From the results embodied in Tables V-VII the following conclusions can be drawn.

1. *Sclerotium oryzae* is parasitic on rice plant and causes Stem-Rot disease under field conditions at Lyallpur (Punjab).

2. The disease is produced whether seed is sown directly or whether seedlings at first grown in uninfected soil are transplanted in land infected with diseased rice stubble or pure culture of the fungus.

The disease is also produced, though to a smaller extent, if seedlings grown in infected soil are transplanted in uninfected soil.

3. The different varieties of rice differ markedly as to their relative susceptibility to the disease.

It may be remarked here that, under Pusa (Bihar) conditions, Mundkur [1935] got negative results of infection experiments in field as well as pots. This is probably due to the difference in climate, nature of soil and other cultural practices between the Punjab and Bihar.

5. Methods of perpetuation of the disease

Observations made in different localities of the province showed that the rice crop grown on land, which had borne a crop affected with *Sclerotium oryzae* the previous year, is attacked by the disease. To test this the following experiments were arranged both at Lyallpur and Kala Shah Kaku.

I. Experiments at Lyallpur

In the first and third plots referred to above under infection experiments (4, III), no further inoculum was added after harvesting the crop in 1934.

In 1935 rice variety No. 349 was grown in these plots by sowing disinfected seed in one half on 1st June and transplanting six-weeks old seedlings raised in uninfected soil in the second half on 15th July. Similar sowings were also done in a plot which had carried a healthy rice crop the previous year to serve as control. The results are given in Table VIII.

Table VIII

Results of an experiment of growing rice in naturally infected land at Lyallpur in 1935

Treatment	Seed sown			Seedlings transplanted		
	Total No. of plants	Infected plants	Per cent infection	Total No. of plants	Infected plants	Per cent infection
Infected land (Diseased crop in 1934)—						
Plot I	144	53	36.8	78	38	48.7
Plot II	109	41	37.6	90	45	50.0
Plot III	96	45	46.8	83	28	33.7
Plot IV	128	8	6.2	87	6	6.9
Plot V	116	11	9.5	86	8	9.3
Total	593	158	26.6	424	125	29.5
Healthy land	531	17	3.2	402	4	1.0

II. Experiments at Kala Shah Kaku

A field in which there were about eighty per cent diseased rice plants showing acute Stem-Rot in 1932 was reserved for the experiment. In 1933 seedlings of nineteen varieties were transplanted in this field. Again in 1934 the same nineteen varieties were grown in this field by transplanting seedlings. The seedlings were raised in an apparently healthy soil, but as the disease is widely present at the farm, freedom of the nursery bed from infection cannot be guaranteed. For the same reason the control plot could not be arranged. The results are given in Table IX.

It is clear from the results given in Tables VIII and IX that if rice is grown in land which carried a diseased crop the previous year the disease is produced.

Table IX

Results of experiments of growing rice in naturally infected land at Kala Shah Kaku, district Sheikhupura, in 1933 and 1934

Serial No.	Variety	1933			1934		
		Total No. of plants	No. of infected plants	Percentage infection	Total No. of plants	No. of infected plants	Percentage infection
1	14	740	589	80·0	829	798	96·6
2	225	561	356	63·5	660	448	68·0
3	46	729	90	12·5	573	281	49·5
4	349	735	545	74·0	823	656	79·8
5	346	812	554	68·0	659	557	84·6
6	360	769	445	60·0	670	587	87·6
7	22	555	111	20·0	662	227	34·3
8	370	805	52	6·4	593	118	20·0
9	3/30	835	74	8·9	659	544	82·5
10	337A	435	237	54·5	599	441	73·6
11	41	761	297	39·0	642	329	51·2
12	7	722	50	7·0	658	134	20·3
13	202	582	367	63·0	654	476	72·7
14	62	628	34	5·4	670	174	26·0
15	12 (S)	707	454	64·0	647	601	92·7
16	278	664	604	91·0	897	786	87·6
17	K. V. 22/28	771	201	26·2	658	348	52·1
18	K. V. 21/28	596	17	3·0	544	348	64·0
19	K. V. 38A/28	289	14	5·0	452	162	35·8

A comparison of Table IX with Tables V-VIII shows that the incidence of the disease is much higher at Kala Shah Kaku than at Lyallpur. This may be due to differences in the nature of the soil, etc., between the two places.

6. Summary

1. Symptoms of the disease of rice caused by the fungus *Sclerotium oryzae* have been described. The most important symptom of the disease in the Punjab is Stem-Rot.

2. The disease is common in many rice-growing districts of the Punjab and causes considerable damage.

3. Inoculation experiments have been carried out in pots as well as in the field. These prove that *Sclerotium oryzae* is parasitic on rice plant both under pot and field conditions in the Punjab.

The disease is produced whether seed is sown directly or whether seedlings at first grown in uninfected soil are transplanted in land infected with diseased rice stubble or pure culture of the fungus.

The disease is also produced, though to a smaller extent, if seedlings grown in infected soil are transplanted in uninfected soil.

4. Rice grown on land which carried a diseased crop the previous year gets diseased.

5. Varieties of rice differ markedly as to their relative susceptibility to the disease.

7. Acknowledgments

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PHOTO-CHEMICAL OXIDATION BY PLANT MATERIALS

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INTRODUCTION

In course of investigations on photo-chemical oxidations of ammonia and similar nitrogenous compounds, Dhar and his co-workers have shown that many inorganic compounds like titania, zinc oxide, etc., act as photo-catalysts. In experiments reported from this laboratory [Fazal-ud-Din, 1935], organic constituents of the soil were found to exert an oxidizing influence. Ordinarily one would expect the organic matter to exert a reducing influence, therefore the oxidizing influence of the organic residues, however small, is of interest in the proper understanding of these processes in soil. It was further found that fresh plant materials also exerted similar influence. The absorption of nitrogen in the form of ammonia by most of the plants has been mainly considered improbable because the mechanism of the oxidation of ammonia inside the plant tissues is not explainable by present-day conceptions of plant physiology. The experiments carried out in this laboratory throw some light on how this oxidation can be brought about in the plants. Further the formation of proteins by interaction of the oxidized nitrogen and sugars has been demonstrated in presence of oxidizing catalyst, namely, titania by Eggleton [1935] and Dhar *et al.* [1934] *in vitro*. If the formation of proteins by similar interaction be postulated as occurring *in vivo*, the role of titania in the experiment *in vitro* can be easily assigned to particular organic plant catalysts in the similar transformation *in vivo*, as both these act as similar oxidizing photo-catalysts.

In course of these experiments it was found that the organic matter derived from various sources and from varied plants, fresh as well as decayed, exerted an oxidizing influence on ammoniacal compounds. The scope of the present investigation has been limited to a detailed examination of the plant tissues of one crop only. Berseem (*Trifolium alexandrinum*) was chosen for these studies.

METHOD

Fifty c.c. of one per cent mono-ethylamine solution and the desired amount of the catalyst were put in Pyrex conical flasks of 300 c.c. capacity. The flasks were plugged with cotton wool and exposed to the sun. In case the flasks were to be sterilized, mono-ethylamine was added after sterilization with a sterile pipette. Nitrous nitrogen was estimated by Griess Ilosvay's colorimetric method.

(1) Fully grown plants were up-rooted. These were washed freely first with tap water, and then with distilled water. The air-dried plants were ground and passed through two mm. sieve. The catalytic power of 0·5 grm. of the ground material was determined. The flasks were not sterilized. Dark controls were kept side by side with the exposed flasks. The results are given in Table I.

TABLE I

Showing the catalytic action of powdered berseem

Serial No.	Treatment	Mg. nitrous nitrogen per 100 c.c.
1	Exposed with the catalyst	0.065
2		0·065
3	Unexposed with the catalyst	*Nil*
4		*Nil*
5	Exposed without the catalyst	0.004
6		0.006

(2) To investigate whether the catalytic principles were heat-stable, half a gram of the material was taken in each of the four flasks containing fifty c.c. of water. Two flasks were heated in an autoclave for half an hour at 125°C. The catalytic power was determined in the usual way. The results (Table II) showed that these catalysts were heat-stable and that the oxidation observed was not due to the enzymatic activities of substances present in the plant material and other bacterial decomposition.

TABLE II

Showing the action of heat on the catalytic action of berseem

Serial No.	Treatment of the catalyst	Mg. nitrous nitrogen per 100 c.c.
1	Unheated	0.078
2	"	0.068
3	Heated	0.065
4	"	0.065

In order to find out whether the catalytic power was confined to a particular tissue or more diffused in nature, the parts like leaves, stems, and roots were separately sampled and their catalytic power was determined. Further, these samples were separately extracted with ether in Soxhlet extraction apparatus for twelve hours. The residues were further extracted with cold and hot water successively. The catalytic power of the different tissues and their extracts corresponding to 0·5 grm. of the substance was determined. The results are given in Table III.

TABLE III

Showing the catalytic action of different tissues and their extracts

Serial No.	Treatment	Mg. nitrous nitrogen per 100 c.c.		
		Roots	Stems	Leaves
1	Untreated	0.069 0.049	0.033 0.043	0.053 0.072
2	Ether extract	0.016 0.016	0.014 0.020	0.016 0.016
3	Cold water extract	0.039 0.039	0.014 0.017	0.039 0.039
4	Hot water extract	0.017 0.017	0.007 0.007	0.020 0.020

These show that :—

1. The catalytic power of the leaves and roots was more than that of stem.
2. The ether extracts of all the tissues were catalytic to the same extent.
3. The water extracts were found to be much better catalysts.
4. It appeared that the catalytic agents were almost completely extractable, as the total oxidation by the three made up the oxidation by the untreated material.

Crude fibre showed very slight catalytic action ; pure cellulose prepared from cotton wool did not show any.

As most of the catalytic power was confined to the water extracts, it was considered that the role of soluble organic materials may be of importance in this process. Therefore the catalytic power of different carbohydrates was determined. The results (Table IV) showed that fructose, glucose and maltose acted as positive catalysts.

TABLE IV.

Showing the catalytic action of the carbohydrates

Serial No.	Catalyst	Mg. nitrous nitrogen per 100 c.c.	
		N/5 ammonia	One per cent mono-ethylamine
1	Fructose	Trace	0.023
		,,	0 019
2	Sucrose	Trace	0.007
		,,	0.007
3	Glucose	0.004	0.013
		0.005	0.019
4	Maltose	0.005	0.034
		Trace	0.036
5	Starch	,,	0.008
		,,	0.008
6	Control	,,	0.008
		,,	0.008

For further confirmation of the catalytic action of glucose, five grams of starch was taken in each of the two flasks. One hundred c.c. of water was added in each. In one flask two c.c. of extra-pure hydrochloric acid was added. Both the flasks were heated for half an hour in an autoclave at 120°C.

Twenty c.c. of the emulsion was titrated with phenolphthalein as an indicator, and the amount of soda required to neutralize the acid was determined. The catalytic power of one gram of the hydrolysed starch was determined after neutralization of the free acid with sodium hydroxide. In the control flasks the equivalent amount of sodium chloride was added.

TABLE V

Showing the catalytic action of hydrolysed starch

Serial No.	Catalyst	Mg. nitrous nitrogen per 100 c.c. solution
1	Starch+sodium chloride	0.010
		0.010
2	Hydrolysed starch+sodium chloride	0.052
		0.055
3	Without any catalyst	0.005
		0.005

The results given in Table V showed that such strong reducing agent as glucose acted as positive oxidizing catalyst and that this was conclusively shown

to be due to the sugar and not to any microscopic impurity that may have been present in the extra-pure sugar employed.

The role of the green colouring matter of the plants in the oxidation process was further studied. For this purpose 0·1 grm. of pure preparations of crystalline and amorphous chlorophylls were used separately for determining the catalytic action. The results showed that both the substances had considerable catalytic power (Table VI).

TABLE VI

Showing the catalytic action of chlorophyll

Serial No.	Catalyst	Mg. nitrous nitrogen per 100 c.c.
1	Amorphus chlorophyll (phytyl)	0.071 0.064
2	Crystalline chlorophyll (ethyl)	0.064 0.052
3	Without the catalyst	0.005 0.005

The catalytic power of the tissues of berseem was unaffected by the age of plants as tissues derived from plants growing for different lengths of time gave almost the same catalytic value. The seeds of berseem plants however were found to have a slightly stronger catalytic action than that of other tissues.

Further it was considered likely that carbon dioxide may be evolved during the course of photo-chemical reaction, therefore the effect of carbon dioxide on this process was investigated. For this purpose four c.c. and eight c.c. of pure carbon dioxide gas was introduced in 300 c.c. flasks containing fifty c.c. of *N*/5 ammonium sulphate solution and one gram of zinc oxide and sealed off. The results (Table VII) showed that carbon dioxide had considerable depressing effect.

TABLE VII

Showing the effect of carbon dioxide on the oxidation of ammonium sulphate

Serial No.	Treatment	Mgs. of nitrogen oxidised per 100 c.c.	
1 2	Control	6.4 9.3	7.8
3 4	4 c.c. CO_2	5.9 5.1	5.5
5 6	8 c.c. CO_2	3.4 2.2	2.8

It has been observed that nitrogen can be assimilated in the ammoniacal form by plants under certain conditions. Eggleton [1935], while studying the transformation of various forms of inorganic nitrogen applied to the soil, found that the amount of nitrite present in the grass leaves increased two hours after the application of ammonium sulphate to the soil. This showed that the ammonium slats were oxidised in the plant. The results reported in this paper indicate how such oxidation is possible by photo-chemical means.

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ABSTRACT

An account of the occurrence of *Chrysomphalus aurantii* Mask. and *Laccifer lacca* Kerr. on grape fruit in Ranchi District, Chota Nagpur, with a note on the *Chalcidoid* parasites of *Aspidiotus orientalis* Newst. P. M. GLOVER (*Journal of the Bombay Natural History Society* 38, 151 : August 1935.

The host plants of *Chrysomphalus aurantii* in India are listed, of these a number are of economic importance, although *C. aurantii* is a serious pest in many parts of the World, it is not of any great economic importance in this country.

C. aurantii and the lac insect, *Laccifer lacca*, were both observed parasitic on young grape fruit (*Citrus decumana* var. *grandis*) grafts in the Ranchi District, Chota Nagpur,—this is an example of lac as a pest. Of the sprays used to control *C. aurantii*, a kerosene-soap emulsion whose preparation is described was the most effective, a resin spray proved satisfactory. Lime-sulphur is not recommended as it scorched foliage severely.

A short description of the female scale is given, and certain differences from the normal in the pygidium of the female insect are described, believed to be local variations. Of these that of greatest interest is that the external paraphyse of the second lobe is greatly reduced and may be absent, and that a distinct club-shaped thickening of the cuticle of the dorsal surface arises from the base of the third incisura, whose structure is exactly that of a paraphyse, but whose disposition is abnormal. The pygidium is figured.

The *Aphelinid Aphytis chrysomphali* Mercet was taken resting on a twig infected with *C. aurantii*, this species was also bred from *Aspidiotus orientalis*. This is thought to be first record for India. The *Encyrtid Comperiella bifasciata* How. is a common parasite of *A. orientalis* and is recorded from India for the first time, and is the second species of the genus known to occur in this country, the other being *C. indica* Ramkrishna Ayyar, other *Chalcidoidea* bred from *A. orientalis* were *Physcus* sp. (near *flaviventris* How.) rarely ; *Tetrastichus purpureus* Cam. as a primary and probably also as a secondary parasite, and *Marietta javensis* How. as a hyperparasite of primary *Chalcidoid* parasites of the *Coccid.* (*Author's abstract*)

MYCOLOGICAL NOTES

A DISEASE OF *CYPERUS TEGETIFORMIS* ROXB. CAUSED BY *KAWAKAMIA CYPERI* (MIYABE AND IDETA) MIYABE

BY

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Imperial Institute of Agricultural Research, Pusa

(Received for publication on 30th March 1936)

(With Plate LV)

Cyperus tegetiformis Roxb. is found in low and wet places throughout Bengal, Assam and Bihar, flowering during the rains. It also occurs in China and Japan, and has been cultivated in the Southern States of America. In Bihar it is collected as a fodder, and for some years past a diseased condition of its twigs and leaves has been noticed during the rainy season.

In the early stages the affected culms are bright yellow and with the progress of the disease they become reddish brown. Finally the cells in the infected tissues collapse and the culms wilt. Transverse sections of the affected parts show extensive unseptate mycelium ramifying in the tissues both intra—and intercellularly. Oospores are also occasionally observed. Incubation of affected plants in a damp chamber leads to rapid growth of the cobwebby mycelium, and to development of conidia.

The conidia have a prominent beak, and a peculiar cell which persists as an appendage or pedicel. The beak very easily breaks off leaving the spore with an abrupt flattened apex, and it is rather difficult to get a conidium with the beak attached. The conidia without the beak are 30-70μ in length and 20-35μ in width, the mean being 49$\mu \times 21\mu$. These measurements are based on the examination of 200 conidia; the beak was found attached in only fifty-six conidia, and the range of the length was 3-15μ and the mean 5·3μ.

The fungus is that described by Miyabe from Japan as *Kawakamia Cyperi* [Miyabe, 1904].

It has also been reported as a serious disease by Patterson [1910] in Texas and South Carolina, where *Cyperus* was introduced for the manufacture of matting.

Miyabe gives the maximum size of conidia as $68 \times 32\mu$ whereas Patterson obtained conidia up to $130 \times 30\mu$. The Pusa strain agrees more closely in size of

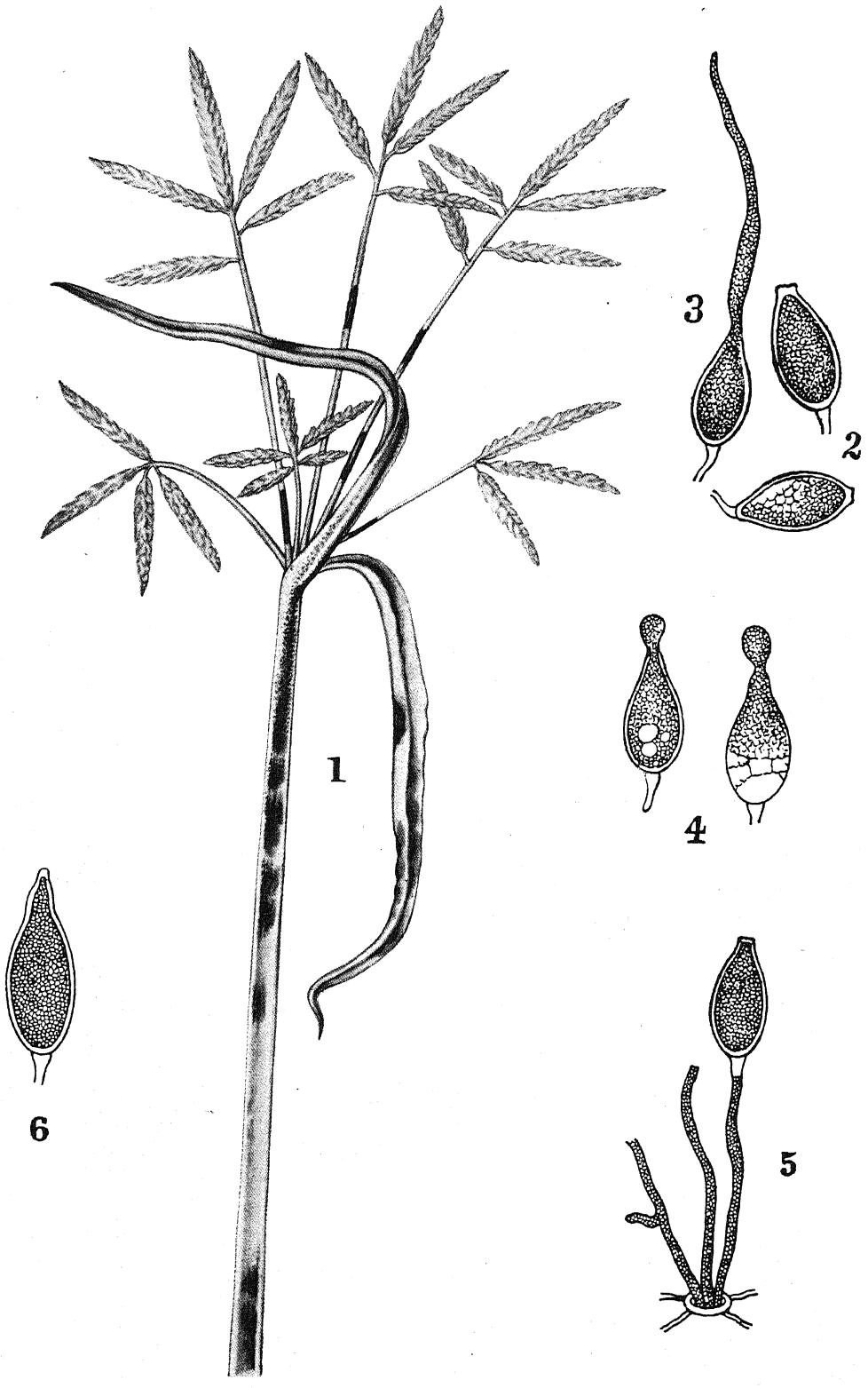

FIG. 1. *Kawakamia* on *Cyperus*

FIG. 2—6. Details of conidia (enlarged).

conidia with the measurements from Japan. Comparison with a herbarium specimen from Texas, however, shows that in general symptoms produced it agrees with the Texas strain.

Fitzpatrick [1930] considers that there is not sufficient reason for separating *Kawakamia* from the genus *Phytophthora*. Wilson [1914], on the other hand, considers it most closely allied to *Basidiophora*. The principal arguments for differentiating *Kawakamia* as a separate genus are the presence of the characteristic, large pedicel and the inability of the fungus to grow on culture media.

This is the first record of *Kawakamia Cyperi* from India.

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A RHIZOCTONIA ON SWEET POTATOES IN BOMBAY

BY

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(Received for publication on 29th April 1936)

(With Plate LVI)

In February 1936 the writer observed in a road-side shop at Kodibag, near Karwar in the Bombay Presidency, several sweet potatoes, *Ipomoea batatas* Poir., that were apparently suffering from disease. The fleshy roots were very much reduced in size and shrivelled and on the surface of their skin, which was red, there were two, three or sometimes four large sclerotia-like bodies such as are seen on the surface of Irish potato tubers suffering from Black Scurf. An examination later of the sweet potatoes offered for sale in the Karwar market indicated that the disease was rather common and that these fetched a very poor price. They came from the neighbouring villages but more information could not be gathered.

With a view to seeing whether the disease was common in the other parts of the Presidency, a visit was paid to the Null bazaar and Crawford markets in the city of Bombay. The sweet potatoes offered for sale were of the white-skinned variety but none of them showed the suspected sclerotia. Whether the roots had been graded and only the better ones sent to the city could not however be ascertained.

The sclerotia when sectioned showed rectangular cells whose walls were thickly incrusted with a black substance. They were rinsed in alcohol, flamed and placed on agar slopes. Hyphae became perceptible in the culture tubes on the third day and profuse mycelial growth on the sixth. After the tenth day, sclerotial formation had started.

The mycelium is light brown, silky, and grows on the walls of the tube. The young hyphae branch freely and fusing hyphae are frequently noticeable. The branches are, as a rule, acute-angled, constricted as they leave the parent hypha and the septum is a little beyond the place of origin. The cells of the hypha are subhyaline, 5 to 10μ in thickness and about 50 to 176μ in length. Sclerotia form slowly in culture, and they are irregular in shape and size. They are addressed, irregularly round, and several of them may coalesce to form a crusty, brittle stromatic mass. They have to be soaked in lacto-phenol for several days before they can be sectioned.

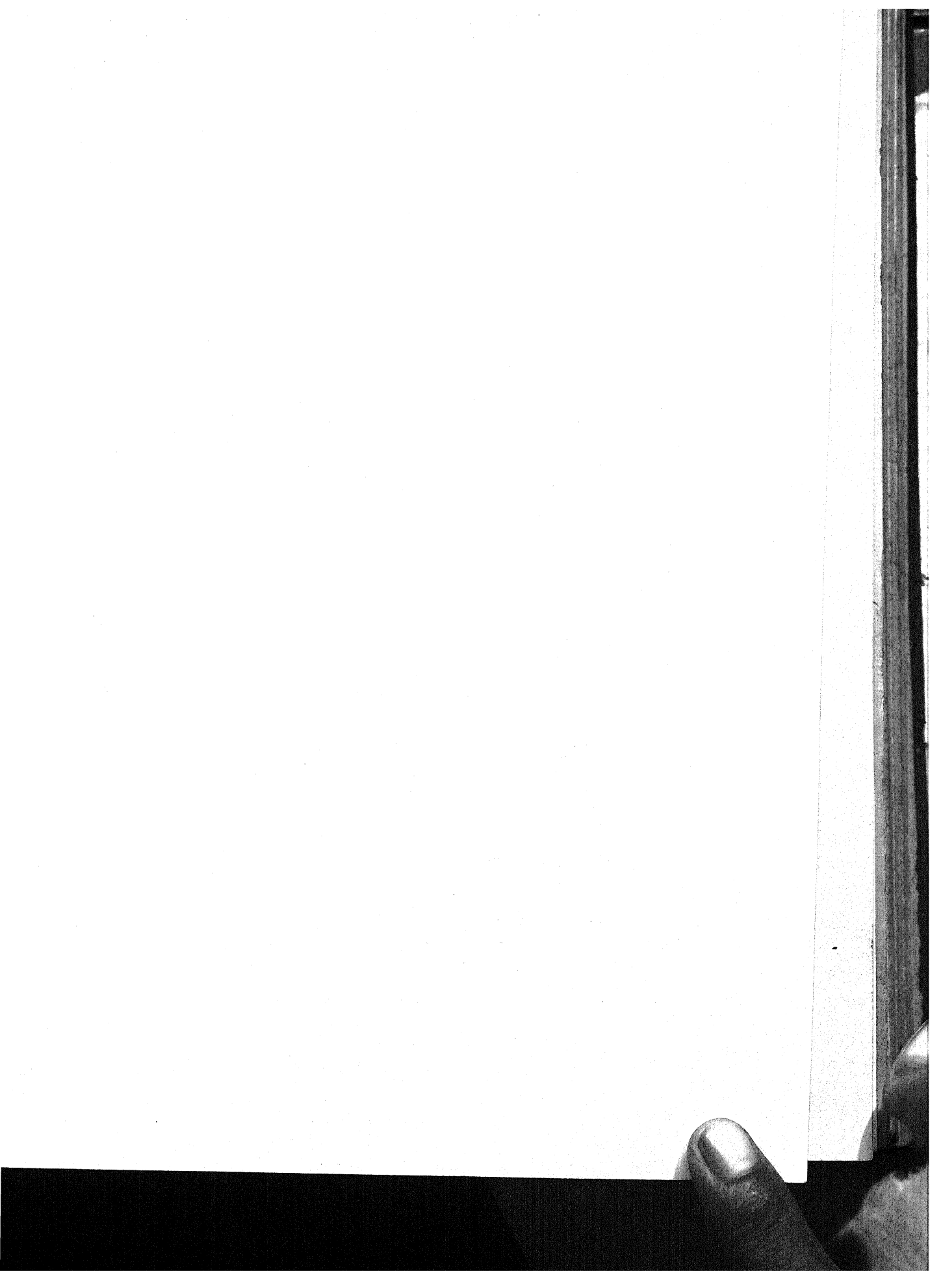
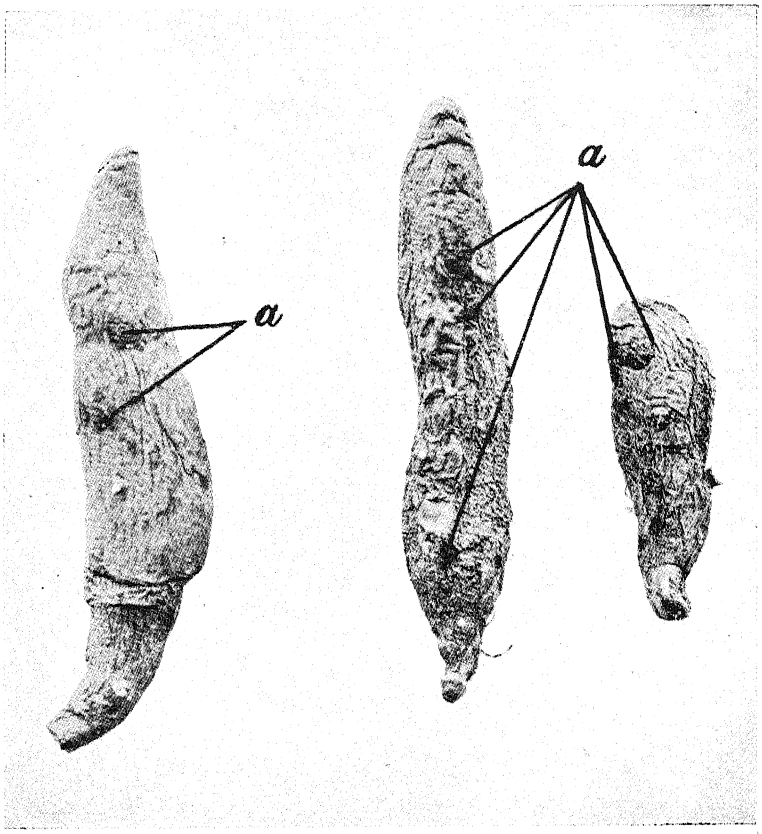


PLATE LVI



Rhizoctonia solani on sweet potatoes
a—Sclerotia.

The above characters indicated that the fungus is a strain of *Rhizoctonia solani* Kuhn (*Corticium vagum* B. and C.). When compared with cultures of *R. solani* that were in the laboratory, one of which had come from Dr. H. H. Whetzel and another from Mr. S. F. Ashby, no doubt was left regarding the identification.

A *Rhizoctonia*-rot of sweet potato plants has been known for some years in the U. S. A. and Harter and Weimer [1929] mention that sclerotia have been found on the surface of the fleshy roots. How far the sweet potato plants are also affected here by this fungus, it was not possible for the writer to ascertain as there was no crop in the villages round Karwar at the time.

Rhizoctonia solani has not so far been reported on sweet potatoes in India as neither Butler and Bisby [1931] nor Uppal, Patel and Kamat [1935] mention it on this host. In addition to making the sweet potatoes unsaleable, it is possible that the fungus may cause damage to the crop as well.

Diseased specimens of the fleshy roots have been deposited in the mycological herbarium of this Institute. Plate LVI shows three of them with sclerotia on the surface, the position of the sclerotia being marked "*a*".

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REVIEW

A comparative study of certain morphological characters of sugarcane × sorgo hybrids. B. A. BOURNE. (*Journal of Agricultural Research*, Vol. **50**, No. 6 : 15th March 1936).

The article gives in some detail the morphological and other characters exhibited by certain intergeneric hybrids between sugarcane and sorghum raised in Florida during 1931-32 and 1932-33.

The studies were made on a population of 128 hybrid plants with the sugarcane variety P. O. J. 2725 as the mother parent ; the male parents employed included (1) Taxas Seeded Ribbon, (2) Early (Kansas) Orange Sorgo, and (3) Sugar Drip Sorgo. The studies reported were chiefly on hybrids with Texas Seeded Ribbon Sorgo.

A rather interesting observation in this article is that " sugarcane stigmas in India have an osmotic pressure very much nearer the optimum for sorgo pollen germination than in either Hawaii or ..... Florida ". This, according to the author, explains the greater ease with which the intergeneric cross between sugarcane and sorghum has been carried out at Coimbatore in India. To ensure success under Florida conditions the sugarcane arrows were covered with a bag from late evening till about 10 A.M. on the succeeding day to prevent reduction of osmotic pressure in the sugarcane stigmatic fluid from rain or dew.

The hybrid plants were found to be intermediate between the two parents in leaf-width and stem-diameter, while in height their average was significantly less than that of either parent. The bud groove on the internode, so characteristic of sorghum, was evident in quite a large number (41 out of 47) of the hybrids. In the inflorescence the sorghum blood was indicated by the frequent presence of the awned IV glume. The hybrid inflorescences exhibited characters, not present in either of the parents such as multiple ovaries and multiple stigmas. The hybrid spikelets were often found to be larger in diameter than those of sugarcane—an obvious influence of sorghum blood. The study includes relative widths of epidermal cells, the distribution of the characteristic short cell groups, the relative number of stomata on the stem epidermis and the presence and distribution of cork and silica cells. The results recorded are of considerable botanical interest. No indication is given of the juice qualities of the hybrids.

The first report of intergeneric hybridization between sugarcane and sorghum was from Coimbatore in 1930. Since then such hybridization has been successfully

effected in Hawaii, Mauritius, Florida and Java. As mentioned by the author, the morphological characters exhibited by the hybrids in Florida are very similar to those described from Coimbatore. The mother parent employed at Coimbatore during the first years was also P. O. J. 2725, but the father parent was *Sorghum Durra* Stapf. The reciprocal cross with sorghum as mother parent was not successful in Florida as also at Coimbatore. (T. S. V.)

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NCAG—AR/35 Annual Report of the Imperial Council of Agricultural Research for the year 1934-35.

7. Review of Agricultural Operations in India

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NCAG—R 1/31 Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8*s*. 3*d*. (Annas 10.)

Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12 or 9*s*. 6*d*.

Review of Agricultural Operations in India, 1933-35. (*In the Press*.)

8. Proceedings of the Board of Agriculture and Animal Husbandry

Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s*. 6*d*.

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The Production of Cigarette Tobacco by Flue-curing, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Pusa Bull*. No. 187. Reprinted (1935). Price Re. 1 or 1*s*. 9*d*.

A Handbook of Statistics for use in Plant Breeding and Agricultural Problems, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S., I.A.S. (*In the Press*.)

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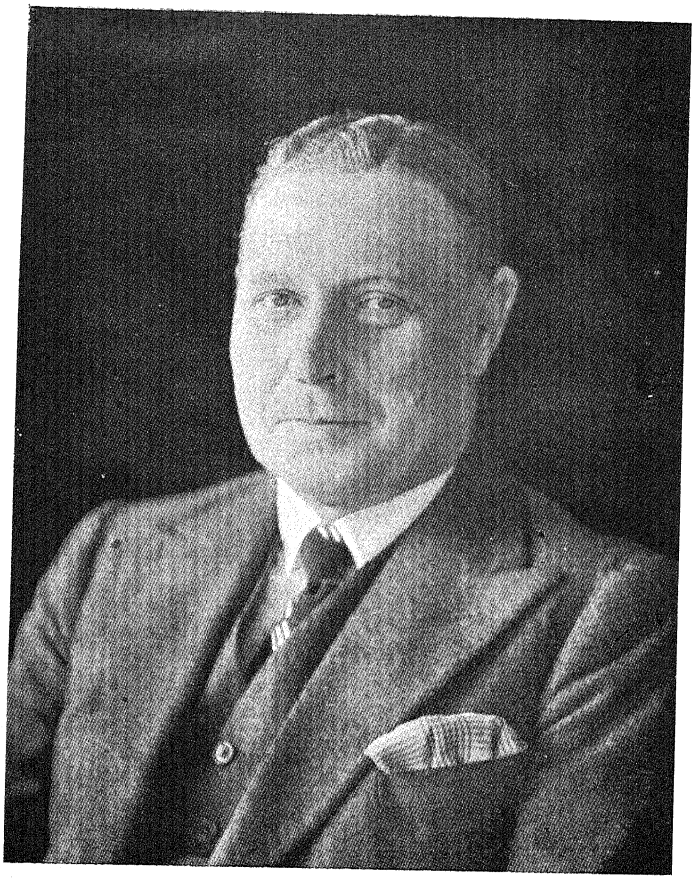

F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S., I.A.S.

F. J. F. SHAW, C.I.E., D.Sc., A.R.C.S., F.L.S., I.A.S.

OBITUARY

We regret to announce the death of a member of our Editorial Committee, Dr. F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S., I.A.S., Officiating Agricultural Expert, Imperial Council of Agricultural Research, who was the permanent Director of the Imperial Institute of Agricultural Research. The Indian Agricultural Service has lost one of its most distinguished and valued members. Dr. Shaw died at Pusa on the morning of July the 29th. He had gone there on tour to advise regarding some final details of the transfer of the Institute to its new building at Delhi.

Dr. Shaw entered the Indian Agricultural Service in 1910 and was first posted to the Imperial Institute of Agricultural Research as Supernumerary Mycologist. Except for the short time spent at Coimbatore as Mycologist to the Madras Department of Agriculture, he spent the whole of his service at the Pusa Research Institute where he became the Second Mycologist and eventually succeeded Sir Albert Howard as Imperial Economic Botanist when the latter became Director of the Institute of Plant Industry, Indore. Between 1929 and 1934 he was Joint Director for a considerable period and in the latter year he succeeded Dr. McRae as permanent Director of the Institute.

An all-round Botanist with a broad scientific outlook, Dr. Shaw has a very considerable volume of successful research to his credit and his name will always be particularly associated with the progress made in the improvement of Indian tobacco and various oil-seeds. As a post-graduate teacher he achieved reputation throughout India and many officers occupying responsible positions in Provincial Agricultural Departments owe much to his wise guidance and the training in research technique which he gave them. The planning of the new Institute at New Delhi was almost entirely his work and, though he had the satisfaction of seeing the completion of the buildings and of the arrangements for transfer, it was not given to him to enter into residence at the new Institute with which his name will be for ever associated.

ERRATA

The Indian Journal of Agricultural Science, Volume VI, Pt. V, October 1936

Page 1024, line 18, *for* 'semi-concious' *read* 'semi-conscious'.

Page 1027, Table, line 11, *for* 'containig' *read* 'containing'.

Page 1029, line 16, *for* 'desposited' *read* 'deposited'.

Page 1106, last line, *for* 'A. J. L.' *read* 'A. J. I.'.

Page 1129, Table IV, last row of columns 4, 7 and 10, *for* '10—6' *read* '10^{-6}' and last but one row of column 7, *for* '$\varkappa$' *read* '$\varkappa^2$'

Page 1130, line 15, *for* 'palnt' *read* 'plant'.

Page 1159, line 21, against price of '*Review of Applied Entomology*', 'A' should be below 'Series' and above '30 *s.*' and 'B' should be below 'Series' and above '15 *s.*'.

ORIGINAL ARTICLES

STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

VIII.* INFLUENCE OF CARBON DIOXIDE ON DEVELOPMENT OF BLACK PIGMENTATION IN *SCHISTOCERCA GREGARIA* FORSK

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(Received for publication on 5th May 1936)

(With Plates LVII-LIX)

CONTENTS

		PAGE
I.	INTRODUCTION	1006
II.	MATERIAL AND METHOD	1006
III.	OBSERVATIONS	1007
	A. Experiment I B. Experiment II C. Experiment III } at variable room temperature (average 30°C.)	1008-9
	D. Remarks on experiments I, II and III	1009
	E. Coloration of V stage hopper at 30°C.	1016
	F. Experiment IV, at 40°C.	1017
	G. Coloration of V stage hopper at 40°C.	1020

*Studies on *Schistocerca gregaria* Forsk.—

I. The Micropyle in *Schistocerca gregaria* Forsk. and some other Acridiidae. *Ind. J. Agric. Sci.* **3** : 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6** : 188-262.

III. Why Locusts Eat Wool. A Study in the Hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6** : 263-67.

IV. Colour Changes and Sexual Behaviour in Desexualised *Schistocerca gregaria* Adults. *Ind. J. Agric. Sci.* **6** : 586-90.

V. Pigmentation and Physical Exertion. *Ind. J. Agric. Sci.* **6** : 591-623.

VI. Influence of Temperature on the Intensity and Extent of Black Pattern in the Desert Locust Hoppers bred Crowded. *Ind. J. Agric. Sci.* **6** : 624-64.

VII. Factors determining the Movement of the Vermiform Larvae. *Ind. J. Agric. Sci.* **6** : 665-71.

PAGE
H. Experiment V, at 44°C. 1021
I. Coloration of V stage hopper at 44°C. 1023
J. Experiment VI, maximum concentration of CO_2 tolerated . 1023
IV. BIOMETRICS 1024
V. DISCUSSION 1028
VI. SUMMARY. 1029
REFERENCES 1030

I. INTRODUCTION

It is well known that *Schistocerca gregaria* Forsk. hoppers show marked colour variations, ranging from light green to dense black with yellow spots. It is held by Uvarov [1928], and others, that this coloration is connected with the 'phases'—*solitaria* and *gregaria*. A typical 'non-swarming' or a *solitaria* hopper is almost spotlessly light green, while a typical 'swarming' or a *gregaria* hopper has extensive black pattern. Intermediate colour grades are not uncommon. In fact the grading, from spotlessly green to intense black, is so gradual that it is impossible, on the basis of coloration, to draw a line between the so-called swarming and non-swarming forms. For this reason some investigators recognise the following forms: *solitaria*, *transiense*, and *gregaria*; and yet others *solitaria*, *dissocians*, *congregans* and *gregaria*. Nor is the coloration of a hopper predetermined or fixed. A non-swarming green hopper, even in the course of a single moult, may acquire the colour compliment of a 'regulation' swarming-type hopper and *vice versa*. Thus the coloration of a hopper, for example in the fifth instar, is practically independent of its coloration at birth. It is well known that the locust hoppers when bred crowded together retain, if born as such, or develop the colour patterns characteristic of the hoppers of a swarm. On the other hand, when bred singly, if born green they do not develop the black pattern, and if born black they lose their colour after a moult or two and finally acquire the green coloration characteristic of a non-swarming phase hopper.

It has been possible, in more ways than one, to induce, in the originally pure green hoppers, the coloration of the swarming-type without resorting to 'crowding'. The production of black pattern in the hoppers bred isolated, but forced to undergo muscular exertion has already been described [Husain and Mathur, 1936]. In this paper it is shown that the black pigmentation of the same pattern as is found in gregarious hoppers in nature, or in hoppers bred crowded, can be developed in hoppers bred isolated, if the concentration of carbon dioxide in the atmosphere of the rearing cage is kept high enough.

II. MATERIAL AND METHOD

Freshly hatched hoppers were reared in cages of fine wire-gauze. Each cage was a cylinder measuring six inches in length and 1·25 inches in diameter. These cages were placed in a glass cylinder (14 inches in height and 8 inches in diameter),

and were arranged along its wall at a distance of about one inch from each other. In the middle and near the bottom of the glass-cylinder, opened the delivery tube of a carbon dioxide generator. A constant current of carbon dioxide was allowed to pass into this cylinder. The mouth of this cylinder was covered over with a card board; thus any excess of air could pass out freely. The concentration of carbon dioxide in the cylinder, three inches above the bottom, was maintained, approximately, at three per cent, by volume. In some of the experiments carbon dioxide was generated by the action of hydrochloric acid on marble. In order to remove all trace of hydrochloric acid fumes from the gas, it was bubbled through water before being introduced into the rearing cylinders. In other experiments commercial carbon dioxide gas under pressure (in cylinders) was used.

Four of the experiments were carried out in the rearing room at an average temperature of 30°C.; and experiments V and VI were conducted in electric thermostats at constant temperatures of 40°C. and 44°C. Each experiment had a control and the hoppers belonging to the same hatching and isolated in similar cages, were reared in similar glass cylinders. Thus the control hoppers and the experimental hoppers were under identical conditions, except for the excess of carbon dioxide in the case of the hoppers under experiments. The food supply, which was renewed thrice a day, consisted of cotton or cabbage leaves, as specified in individual experiments, and this kept the relative humidity high, (80—90 per cent).

III. Observations

Before entering upon the details of the observations made, a word of explanation with regard to the influence of temperature on the intensity and extent of black pigmentation, acquired by the hoppers bred crowded, is necessary. Other factors being constant, the intensity and extent of black pigmentation within certain limits, varies inversely with temperature. At 30°C., for example, the black pigmentation of hoppers bred crowded is very pronounced, at 40°C. it becomes much reduced, while at 44°C., the hoppers are almost whitish.

It may be mentioned here that an isolated hopper reared, in an atmosphere containing three per cent carbon dioxide, at a particular temperature closely resembles, so far as the intensity and extent of black pigmentation is concerned, the hoppers bred crowded at that particular temperature. Thus a hopper reared isolated at 30°C. in an atmosphere containing higher concentration of carbon dioxide, will acquire approximately the same intensity and extent of black pigmentation in the fifth instar as a crowded hopper of that instar at that temperature. Husain and Ahmad [1936] have studied the development of black pigment in crowded hoppers of *Schistocerca gregaria* at certain constant temperatures and have fully described and illustrated (with coloured plates) the coloration of the hoppers at each stage. For this reason it has not been considered necessary to describe the coloration at each stage and the descriptions of typical V stage hoppers only, as obtained under the influence of carbon dioxide, are given.

In what follows the term "*Black type*", refers to that intensity and extent of black pigmentation, which a hopper bred crowded normally acquires at a particular temperature. It must be remembered that a hopper referred to as "*Black type*", say at 40°C., shows the black pigmentation typical of the hoppers reared crowded at 40°C., but may not be as strongly pigmented as a hopper bred crowded at 30°C. In certain cases the intensity of pigmentation, obtained under the influence of carbon dioxide, was even higher than that of the hoppers bred crowded at that temperature. Such cases have been referred to as "*strongly black type*". *Green type* refers to hoppers devoid of all black markings, but the term does not indicate the intensity of the basic green colour. For coloured plates illustrating the two types reference may be made to in an earlier publication [Husain and Mathur, 1936].

A. Experiment I

The hoppers in this experiment were taken from two hatchings.

History.—Reference No. P 54 and P 57.

The solitary phase adults of both the pairs were collected from Pasni (Makrān coast) on 8th August 1932, and were received at Lyallpur on 19th August 1932, and reared in pairs in wire-gauze cages measuring 9-in. × 9-in. × 9-in. Females P 54 and P 57 laid a cluster of eggs each on 31st August 1932. Both the egg pods hatched on 14th September 1932, and the hatchings were pure green, the progeny of P 54 and P 57 consisting of 62 and 64 green hoppers respectively. Thus the hoppers from both these hatchings showed distinct '*solitaria*' tendency.

Conditions.—The hoppers were isolated and bred in an atmosphere containing excess of carbon dioxide, from 14th September 1932 onwards.

Food.—Cotton leaves were provided for feeding and were changed thrice a day.

The temperature of the laboratory where the hoppers were reared was :—

Highest maximum .	. 35°C.	Highest minimum .	. 32°C.
Lowest maximum .	. 29·5°C.	Lowest minimum .	. 25°C.
Average maximum	. 31·8°C.	Average minimum .	. 27·7°C.

Average mean temperature :—29·7°C.

B. Experiment II

History.—Reference No. P 55.

The solitary phase adults were collected from Pasni on 8th August 1932 and were received in the laboratory on 19th August 1932. They were placed in pairs in wire-gauze cages measuring 9-in. × 9-in. × 9-in. The date of egg-laying was not recorded. The hoppers hatched out on 21st September 1932 and the hatching was a mixed one, consisting of fifteen green, ten intermediate and forty-three black hoppers. Only green hoppers were selected for this experiment. These hoppers belonging to a mixed hatching did not have the *solitaria* tendency of the hoppers in experiment I, but when bred in isolation developed marked *solitaria* coloration.

Conditions.—The hoppers were bred in isolation in an atmosphere containing excess of carbon dioxide.

Food.—Cotton leaves changed thrice a day.

The temperature of the rearing room during the experiment was :—

Highest maximum .	. 33·5°C.	Highest minimum .	. 29·2°C.
Lowest maximum .	. 29°C.	Lowest minimum .	. 25°C.
Average maximum	. 31·8°C.	Average minimum .	. 27·8°C.

Average mean temperature :—29·8°C.

C. Experiment III

History.—Reference No. P 53.

The solitary adults were collected from Pasni on 8th August 1932 and were received at Lyallpur on 17th August 1932. They were reared in wire-gauze cages measuring 9-in. × 9-in. × 9-in. The date of egg-laying was not recorded. Hatching was a mixed one, consisting of fifty-six green and five intermediate hoppers. Only green hoppers were taken for this experiment. Thus the *solitaria* tendency of these hoppers was less marked than that of the hoppers used in experiment I, but more than that in the hoppers in experiment II.

Conditions.—The green hoppers were isolated and bred in an atmosphere containing excess of carbon dioxide from 11th September 1932 onwards.

Food.—Cotton leaves changed three times a day.

Temperature of the laboratory during the experiment was :—

Highest maximum .	. 35°C.	Highest minimum .	. 30°C.
Lowest maximum .	. 29°C.	Lowest minimum .	. 25·5°C.
Average maximum	. 31·7°C.	Average minimum .	. 27·6°C.

Average mean temperature :—29·6°C.

D. Remarks on experiments, I, II and III

These experiments were conducted in a room where the temperature during the experiment varied from 25°C. to 35°C. and the mean average was approximately 30°C. This was the lowest temperature tried in this investigation. Thus, as expected, the intensity and extent of black pigmentation acquired by the hoppers in these experiments was the greatest. The progress of black pigmentation acquired from instar to instar by these isolated hoppers under the influence of carbon dioxide, as well as the state of coloration (from instar to instar) of isolated hoppers in the control, reared in an ordinary atmosphere is indicated in Table I.

TABLE I

Progress of pigmentation from stage to stage in hoppers in experiments, I, II and III

Experiment No.	Hatching	Details of experiment	Reference No.	I stage	II stage	II (*a*) stage	III (*b*) stage	IV stage	V stage
I	Pure green	Experimental hoppers bred in carbon dioxide atmosphere	P 54-57/CO_2 A	Green type	Intermediate	Intermediate	Black type	Strongly black type	Strongly black type
			Do. C	Do.	Green	Do.	Do.	Black type	Black type
			Do. D	Do.	Intermediate nearer green type	Intermediate nearer black type	Do.	Do.	Strongly lack type
			Do. E	Do.	Intermediate nearer black type	Do.	...	Do.	Black type.
			Do. F	Do.	Do.	...	...	...	...
		Control hoppers	P54-57/Cont. 1.	Do.	Green type	Green type	...	Green type	Green type
			Do. 2	Do.	Do.	Do.	...	Do.	
			Do. 4	Do.	Do.	Do.	...	Do.	Green type

II	Mixed hatching	Experimental hoppers	P 55/CO_2	A.	Do.	Intermediate	...	...	...	...
			Do.	B.	Do.	Black type	Black type	...	Strongly black type	Strongly black type
			Do.	C.	Do.	Intermediate nearer black type	Intermediate nearer green type	...	Black type	...
			Do.	D	Do.	Black type	Black type	Black type	..	...
			Do.	E.	Do.	Do.	...	...	.	...
		Control hoppers	P 55/Cont.	F.	Do.	Green type	Green type	Green type	...	...
			Do.	G.	Do.	Intermediate nearer green type.	Do.	...	Green type	...
			Do.	H	Do.	Intermediate nearer black type	Do.	...	Do.	...
			Do.	K	Do.	Intermediate nearer black type	Do.	...	Do.	Green type
III	Mixed hatching	Experimental hoppers	P 53/CO_2	1.	Do.	Black type	Black type	Black type	Strongly black type	Strongly black type
			Do.	2.	Do.	Intermediate nearer black type	Intermediate	Intermediate nearer black type	Black type	Do.
			Do.	5.	Do.	Black type	Black type	...	Do.	Do.
		Control hoppers	P 53/Cont.	6.	Do.	Green type	Green type	...	Green type	Green type
			Do.	7.	Do.	Do.	Do.	...	Do.	Do.
			Do.	8.	Do.	Do.	Do.	...	Do.	Do.

Experiment I was started with pure green hoppers of first stage, which were the progeny of '*solitaria*' parents. Whereas the control hoppers remained green throughout the hopper stages, all the hoppers reared in an atmosphere containing excess of carbon dioxide, with the exception of one (P 54-57/CO_2C), were intermediate, *i.e.*, showed some pigmentation, even after the first moult, *i.e.*, in the second stage. During the next stage they were all '*Black type*', and in the fourth and fifth stages all the surviving hoppers were '*Black type*'. Some of them had more intense and more extensive black pigmentation than a typical hopper in a crowded rearing at 30°C. and these forms have been described as "*Strongly black type*" (Plate LVIII, fig. 1). The duration of the life-cycle of these hoppers is given in Table II. All the hoppers in this experiment, which had survived, had an extra moult which we have called III-b*. None of the control hoppers had an extra moult.

TABLE II

Life-history of hoppers in experiment I

Reference No. P 54-57.

Room temperature { Average maximum 31·8°C. / Average minimum 27·7°C.

Days	Dates (1932)	Experimental					Control		
		A	C	D	E	F	1	2	4
1	14th Sept.	I	I	I	I	I	I	I	I
2	15th ,, .								
3	16th ,, .								
4	17th ,, .								
5	18th ,, .								
6	19th ,, .	II		II		II	II	II	II
7	20th ,, .		II		II	Died			
8	21st ,, .								
9	22nd ,, .								
10	23rd ,, .								
11	24th ,, .								
12	25th ,, .								
13	26th ,, .	III(*a*)			III (*a*)		III	III	III
14	27th ,, .		III (*a*)	III (*a*)					
15	28th ,, .								
16	29th ,, .								
17	30th ,, .								
18	1st Oct. .	III (*b*)	III (*b*)	III (*b*)					
19	2nd ,, .				III (*b*)				
20	3rd ,, .								
21	4th ,, .								
22	5th ,, .								
23	6th ,, .		IV						
24	7th ,, .			IV			IV		

* The extra instar is between the normal third and the normal fourth instars. The hopper approaches the fourth instar hopper in size, but resembles the third instar hopper in structure, notably the wing-pads.

TABLE II—*contd.*

Days	Dates (1932)	Experimental					Control		
		A	C	D	E	F	1	2	4
25	8th Oct. .								IV
26	9th ,, .	IV			IV			IV	
27	10th ,, .								
28	11th ,, .								
29	12th ,, .								
30	13th ,, .								
31	14th ,, .	V							
32	15th ,, .						V	Died	V
33	16th ,, .			V					
34	17th ,, .								
35	18th ,, .	V			V				

The hoppers in experiment II were 'green' hoppers from a mixed hatching which had a predominance of 'black' hoppers. The parents were '*solitaria*'. In this case all the control hoppers showed some gregaria tendency in II stage, but in all the subsequent hopper stages they were of '*Green type*'. The experimental hoppers in practically all cases acquired the extent and intensity of black pigmentation of '*Black type*' even in the II stage. During the subsequent stages they were, of course, '*Black type*' (Table I). The duration of their life-cycle is given in Table III.

TABLE III

Life-history of hoppers in experiment II

Reference No. P 55.

Room temperature { Average maximum 31·8°C. / Average minimum 27·8°C.

Days	Dates (1932)	Experimental					Control			
		A	B	C	D	E	F	G	H	K
1	21st Sept. .	I	I	I	I	I	I	I	I	I
2	22nd ,, .									
3	23rd ,, .									
4	24th ,, .									
5	25th ,, .									
6	26th ,, .									
7	27th ,, .	II	II	II	II				II	II
8	28th ,, .						II			
9	29th ,, .					II				
10	30th ,, .	Died (Accident)						II		

TABLE III—*contd.*

Days	Dates (1932)	Experimental					Control			
		A	B	C	D	E	F	G	H	K
11	1st Oct.									
12	2nd ,,		III	II	III(*a*)				III	III
13	3rd ,,						III(*a*)			
14	4th ,,									
15	5th ,,							III		
16	6th ,,									
17	7th ,,									
18	8th ,,		IV							
19	9th ,,			IV			IIIb			IV
20	10th ,,									
21	11th ,,				III(*b*) Died	II Died	Died (Accident)			
22	12th ,,									
23	13th ,,									
24	14th ,,									IV
25	15th ,,									
26	16th ,,							IV		
27	17th ,,		V					(removed.)		
28	18th ,,									
29	19th ,,			V						V
30	20th ,,									

Experiment III was started with pure green hoppers which were selected from a mixed hatching having a predominance of '*Green type*'. The control hoppers remained green throughout their hopper stages, while the experimental hoppers manifested a marked approach to '*Black type*' even after the first moult. One of the hoppers, in the fourth stage and all the hoppers in the fifth stage were described as '*Strongly black type*' (Table I).

Mention may be made of the fact that occasionally, during these experiments, we had hoppers which had a deep brown pattern in place of the usual black pattern. These markings appeared as a diffuse pattern on a base of a lighter tint of the same colour, instead of a well-marked black pattern on a yellowish background (Plate LVIII, fig. 3). The hoppers of this type also occur in crowded rearings where the temperature is low and frequently falls below the developmental zero of the Desert Locust.

The duration of the life-cycle of the hoppers in experiment III is given in Table IV.

TABLE IV

Life-history of hoppers in experiment III

Room temperature { Average maximum 31·7°C. / Average minimum 27·67°C.

Days	Dates (1932)	Experimental		
		1	2	5
1	11th Sept.	I	I	I
2	12th ,,			
3	13th ,,			
4	14th ,,			
5	15th ,,	II	II	
6	16th ,,			II
7	17th ,,			
8	18th ,,			
9	19th ,,			
10	20th ,,	III(*a*)		
11	21st ,,			
12	22nd ,,			III
13	23rd ,,	III(*b*)		
14	24th ,,		III (*a*)	
15	25th ,,			
16	26th ,,			
17	27th ,,			
18	28th ,,		III (*b*)	
19	29th ,,	IV		
20	30th ,,			IV
21	1st Oct.			
22	2nd ,,			
23	3rd ,,			
24	4th ,,	V		
25	5th ,,		IV	
26	6th ,,			
27	7th ,,			V
28	8th ,,			
29	9th ,,			
30	10th ,,			
31	11th ,,			
32	12th ,,			
33	13th ,,		V	

To give an idea of the extent of pigmentation acquired under experimental condition in experiments I, II and III we give below a brief description of a representative fifth instar hopper, described in Table 1 as '*Black type*' :—

*E. Coloration of V stage hopper reared singly in an atmosphere of three per cent carbon dioxide at 30°C.** [Plate LVII].

(*i*) *General colour.*—Black predominates ; basic colour usually pale yellow.

(*ii*) *Head* : *Frons.*—Greenish yellow with fine black dots. Black at carinae.

Clypeus.—Ventral portion dull black, dorsally lighter.

Labrum.—Black.

Vertex.—Deep black on either side of the greyish epicranial suture. Deep black colour running down behind the eyes and joining the deep black sub-ocular genal stripe. Post-ocular area brown to black.

Gena.—Sub-ocular genal stripe deep black and broad. Area behind the stripe yellowish brown.

Antenna.—Black except lateral aspect which is brown.

Mandibles.—Black.

Maxillæ.—Dull white ; palpi black.

Labium.—Dull white ; palpi black ; galea black.

(*iii*) *Cervical sclerites.*—Black.

(*iv*) *Thorax* : *Pronotum.*—Basic colour greenish yellow or yellow ; white along the lower margin and the anterior and posterior margins ; anterior upper half margin often yellow ; keel white behind the post-sulcus and black in front. Entire area in front of anterior sulcus black with yellow specks. This black area runs downwards in front of the anterior sulcus and joins lateral black patch; lateral black patch extends from anterior margin to posterior margin behind and ventral margin below. The dorsal margin of this lateral pattern slanting from anterior sulcus to middle of the posterior margin. There is a black metazonal triangular area separated from the lateral pattern by a small area of pale yellow colour. The extent of this area varies.

Meso-episternum, *Meso-epimeron* } Yellowish brown, black along the margin.

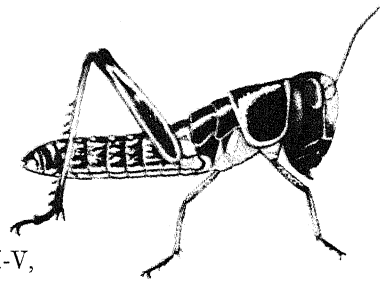
Meta-episternum, *Meta-epimeron* } Black.

Sternum :—Dirty brown with numerous irregular black patches.

(*v*) *Leg* : *I and II pair.*—Dirty white with numerous smoky spots. Trochanter yellow. Femur yellow. Head of tibia and tarsi light brown. Claws brownish.

* For full description reference may be made to *Ind. J. Agric. Sci.* **6**: 624-64.

FIG. 1*b*. Abdominal segments III-V, lateral view.



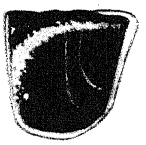
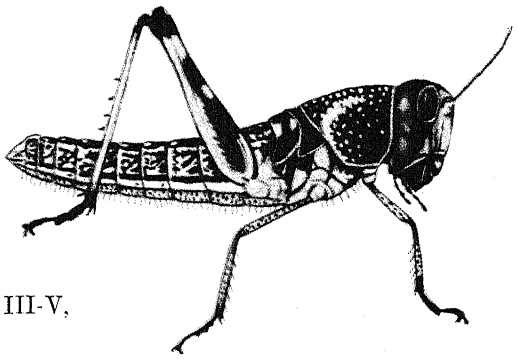


FIG. 1*a*. Pronotum, lateral view.

FIG. 1. II stage hopper reared isolated at 30°C. in excess of carbon dioxide.

FIG. 2*b*. Abdominal segments III-V, lateral view.



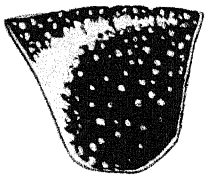


FIG. 2*a*. Pronotum, lateral view.

FIG. 2. III stage hopper reared isolated at 30°C. in excess of carbon dioxide.

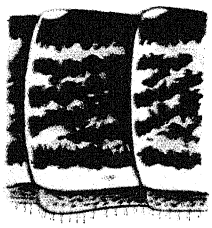
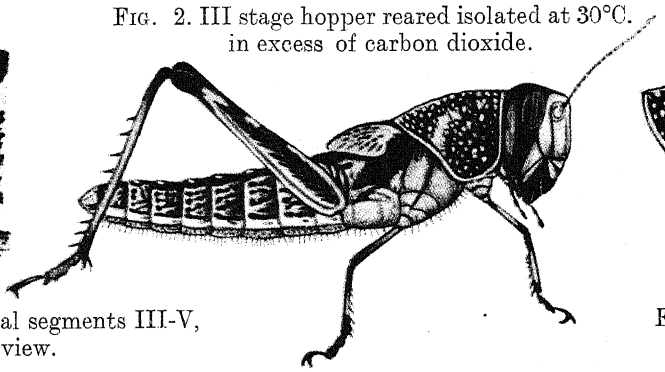


FIG. 3*b*. Abdominal segments III-V, lateral view.



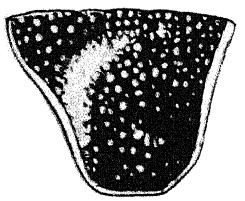


FIG. 3*a*. Pronotum, lateral view.

FIG. 3. IV stage hopper reared isolated at 30°C. in excess of carbon dioxide.

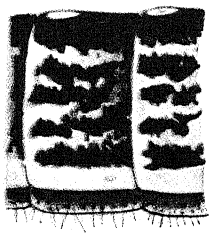
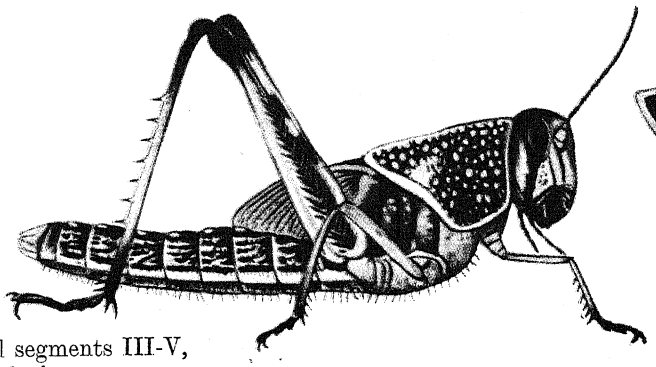


FIG. 4*b*. Abdominal segments III-V, lateral view.



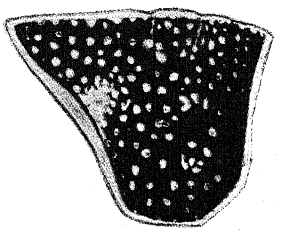


FIG. 4*a*. Pronotum, lateral view.

FIG. 4. V stage hopper reared isolated at 30°C. in excess of carbon dioxide.

III pair—hind femur.—Muscle-impression area from the head of femur to about 7/8 of its length black. Tibia-femur joint black. Spurs black. Tarsi black. Pads light brown. Tibia up to first spine of anterior row light black.

(*vi*) *Abdomen*: *Dorsal streak.*—Black with white triangular areas at the posterior margin of each segment.

Segment I.—Whitish all over.

Remaining segments.—Five black spots along the posterior margin, often fused with streaks in front. The lowermost of these spots fuses with a rather wide ventral straight streak ; in front of the third and fourth there are irregularly arranged spots. The fifth spot runs into the dorsal black streak.

Ventral side.—Yellow with many black dots.

It may be stated that at this temperature there appeared, now and then, some hoppers that had the patterns of brownish black colour with a sort of diffused coloration of a lighter hue (Plate LVIII, fig. 3).

When compared with hoppers bred crowded at 30°C. the pigmentation here is more intense as well as more extensive. The *Black type* representatives of II-V stages reared under these conditions are illustrated in Plate LVII, figs. 1-4.

F. Experiment IV

History.—Reference No. of parents : L 326.

The parents were laboratory bred and paired in the adult stage. The eggs were laid on 18th March 1933 and the hoppers hatched out on 5th April 1933. The hatching was a mixed one and consisted of thirty-eight green and four intermediate hoppers. The hoppers used in the experiment were green. Thus the '*solitaria*' tendency was of the same degree as in the hoppers in experiment III.

Conditions.—The experiment was started on 5th April 1933 at 40°C. in a thermostat. Two of the hoppers (N and P) were bred during the 1st stage under normal atmospheric condition at 40°C. and were transferred to the carbon dioxide atmosphere at the beginning of the second stage. The control hoppers were also reared at 40°C.

Remarks.—Taking into account the high temperature, the extent of black pigmentation in some cases was very marked indeed. The state of black pigmentation in V stage hopper described in Table V as *strongly black type* is illustrated in Plate LVIII, fig. 2.

TABLE V

Progress of pigmentation from stage to stage in hoppers in experiment IV

Reference No.		I stage	II stage	III(*a*) stage	III (*b*) stage	IV stage	V stage
A. L 326/5.IV.33	Experimental	Green type	Intermediate nearer black type	Intermediate nearer black type	Almost black type	Black type	Black type
F. Do.		Do.	Do.	Do	Strongly black type	Strongly black type	Do.
H. Do.		Do.	Do.	Died	...	...	...
N Do.		Do.	Green type *	Black type	Strongly black type	Died	...
P. Do.		Do.	Do. *	Green type	Do.	Black type	Almost black type
J.	Control.	Green type	Green type	Green type	Died	...	...
M.		Do.	Do.	Do.	Green type	Green type	...

* These hoppers were reared in ordinary atmosphere during their first instar.

Table V shows the progress of pigmentation acquired from stage to stage, and the duration of the life-history of the hoppers is given in Table VI. Below is given a brief description of a typical V stage hopper reared at 40°C. in an atmosphere containing three per cent carbon dioxide.

TABLE VI

Life-history of hoppers in experiment IV

Reference No. L.326. Temperature 40°C.

Days	Dates 1933	Experimental					Control	
		A	F	H	N	P	J	M
1	5th April	I	I	I	I	I	I	I
2	6th ,,							
3	7th ,,							
4	8th ,,							
5	9th ,,							
6	10th ,,	II	II	II	II	II	II	II
7	11th ,,							
8	12th ,,			Died				
9	13th ,,		IIIa					
10	14th ,,				IIIa	IIIa	III	
11	15th ,,	IIIa	IIIb				Died	
12	16th ,,							
13	17th ,,							
14	18th ,,	IIIb			IIIb	IIIb		III
15	19th ,,							
16	20th ,,							
17	21st ,,		IV					
18	22nd ,,	IV						
19	23rd ,,							
20	24th ,,				IV	IV		
21	25th ,,				Died			IV
22	26th ,,							
23	27th ,,		V			V		Died
24	28th ,,	V						
25	29th ,,	Died						
26	30th ,,							

G. Coloration of a typical V stage hopper reared isolated in an atmosphere containing three per cent carbon dioxide at 40°C. [*Plate LVIII, Figs. 2, 2a, 2b*].

(*i*) *General colour.*—Black pattern less extensive but brighter than at 30°C. Ground colour bright yellow.

(*ii*) *Head : Frons.*—Yellow, a tinge of green at the frontal carinae.

Clypeus.—Dorsal half bright yellow, ventral half greyish yellow.

Labrum.—Yellowish in the middle, white at the margins.

Vertex.—Black on both sides of the greyish epicranial suture.

Gena.—Yellow and black ; sub-ocular genal stripe black and continued into the brownish narrow area running behind the compound eyes.

Post-gena.—Light brown to light black.

Antennae.—Basal five and distal five segments brownish, remaining segments dorsally smoky and ventrally brownish.

Mandibles.—Teeth black, exposed portion yellowish.

Maxillae, Labium.—Dull white with a tinge of yellow at places.

(*iii*) *Cervical sclerites.*—Strongly smoky.

(*iv*) *Thorax : Pronotum.*—Black with bright yellow dots. Ventral and posterior margins white and run into median metazonal line. Keel white in metazonal region, *i.e.*, behind the hind sulcus, yellow in front. Prozona black, with bright yellow dots. Greenish black patch on the lateral region at base of post-sulcus. Metazonal triangular patches light black with several yellow dots on each side of median line.

Meso-notum, Meta-notum.—Light black stripes on both sides of white median line. Below it brownish black stripes.

Meso-episternum, Meso-epimeron.—Yellow with black faint lines at the anterior margin.

Meta-episternum.—Yellow.

Meta-epimeron.—Yellow with anterior and posterior black margins.

Sternum.—Uniformly yellow.

(*v*) *Leg : I and II pair.*—Femur and tibia yellow. Tarsi light brown. Tips of claws dark brown.

III pair—hind femur.—A narrow black pattern on the muscle-impression area starting from the head of femur to 3/4 its length. Tibia-femur joints brown to black at places.

(*vi*) *Abdomen : I and II segments.*—Uniformly yellow ; no black markings.

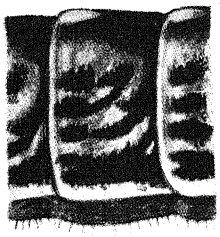


FIG. 1*b*. Abdominal segments III-V, lateral view.

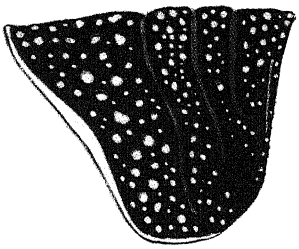


FIG. 1*a*. Pronotum, lateral view.

FIG. 1. V stage hopper, 'Strongly Black Type' at 30°C. in excess of carbon dioxide.

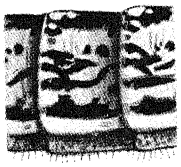
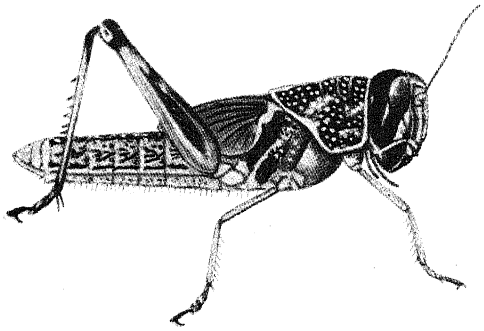


FIG. 2*b*. Abdominal segments III-V, lateral view.



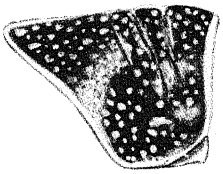


FIG. 2*a*. Pronotum, lateral view.

FIG. 2. V stage hopper 'Strongly Black Type' at 40°C. in excess of carbon dioxide.

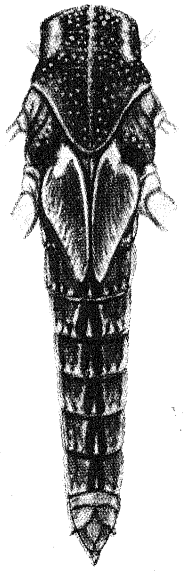


FIG. 3*c*. Thorax and abdomen, Dorsal view.

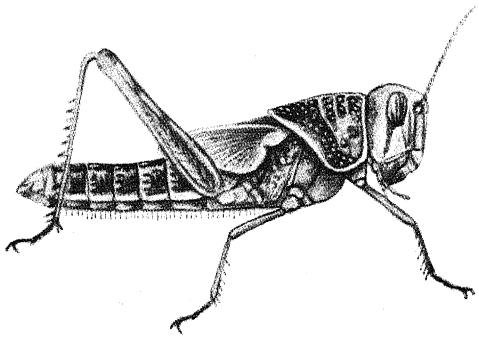


FIG. 3. V stage hopper reared at 30°C. in excess of carbon dioxide (special brownish colour).

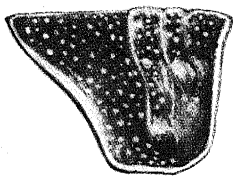


FIG. 3*a*. Pronotum, lateral view.

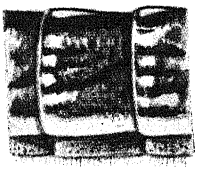


FIG. 3*b*. Abdominal segments III-V, lateral view.

III to VIII segments.—Light black median dorsal streak with white triangular areas at posterior margin of each segment. Five black elongated dots at posterio-lateral margin of each tergum, ventral—most dot is united with narrow light black streak which is fainter anteriorly; in front of second dot a faint brown streak; opposite the third and fourth dots irregular small two or three dots; the fifth dot runs into narrow black dorsal streak.

IX segment.—A faint dorsal and a faint ventral streak.

X segment.—Uniformly yellow.

This colour approximates fairly closely with that of the hoppers bred crowded at 40°C. [*Cf.*—*Ind. J. Agric. Sci.* **6**, 624—64].

H. Experiment V

History.—Reference No. of parents : L 316.

The parents were laboratory bred and were paired in the adult stage.

The eggs were laid on 9th April 1933. The hoppers hatched out on 26th April 1933; the hatching was pure green and consisted of twenty hoppers. The experiment was conducted at 44°C. in a thermostat.

Remarks.—It is at this temperature, which is very near the upper vital limit for the hoppers of the Desert Locust, that a marked difference between the coloration of the hoppers bred crowded and those reared isolated in an atmosphere of three per cent carbon dioxide, occurred.

The hoppers reared crowded at 44°C., in an ordinary atmosphere, show no black pigmentation whatsoever [Husain and Ahmad, 1936]. They are generally white all over the body and it is only rarely that they have a tinge of yellow at places. But under the influence of three per cent carbon dioxide the black pigment appears (Plate LVIII, figs. 1—4). Even the ground colour, particularly in the advanced stages, is light yellow all over. The progress of pigmentation from instar to instar under these conditions is shown in Table VII and the duration of the life-history of these hoppers is given in Table VIII.

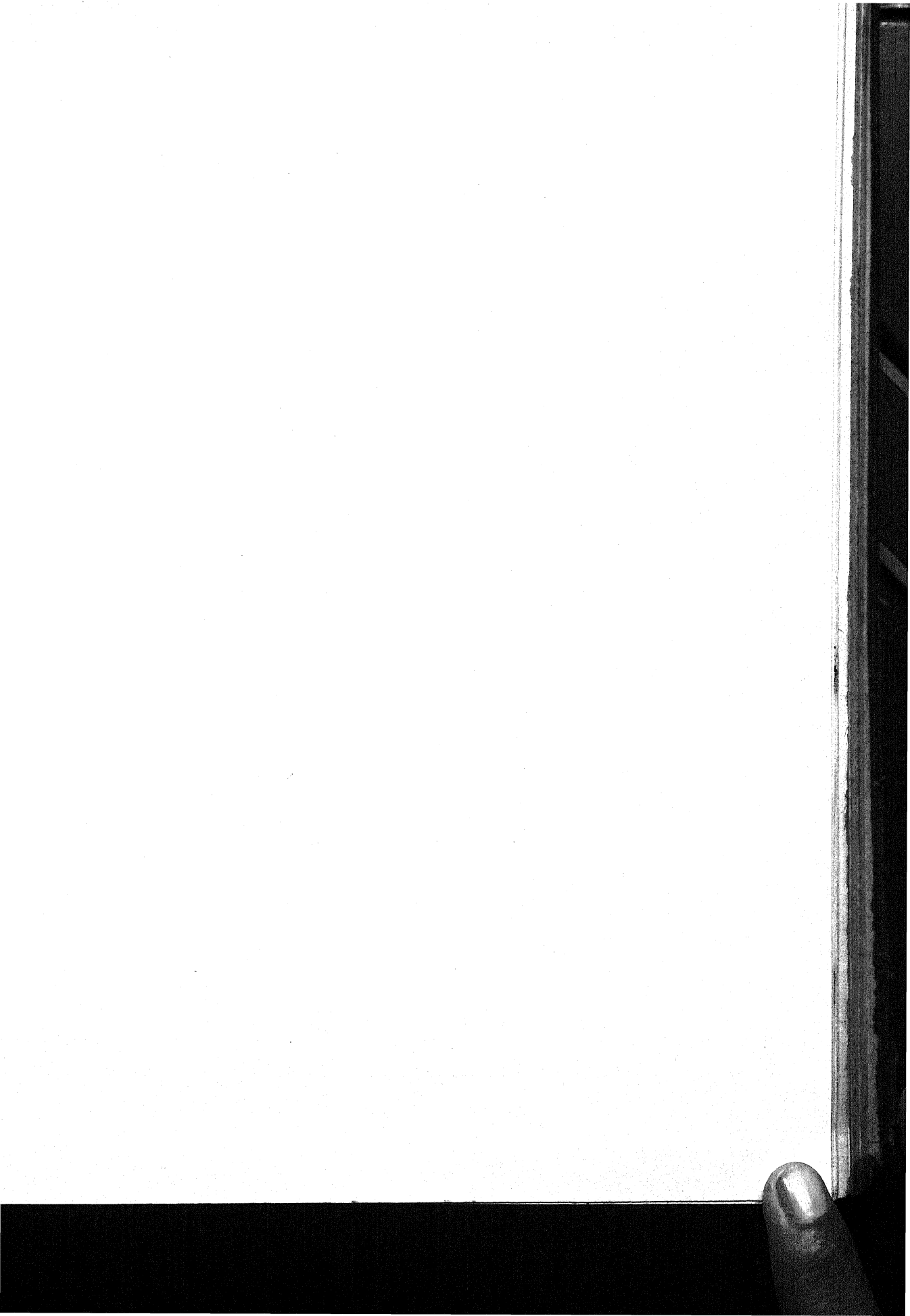
It is necessary to repeat once again that the term black type refers to the coloration developed in the hoppers bred crowded at 44°C.

TABLE VII

Progress of pigmentation from stage to stage in hoppers in experiment V

(Temperature 44°C.)

Reference No.	I stage	II stage	III stage	IV stage	V stage
A. L316/26. iv. 33	Green type .	Intermediate nearer black type	Intermediate nearer black type	Black type .	Back type
C. Do. .	Do. .	Intermediate .	Do. .	Intermediate nearer black type	Do.
F. Do. .	Do. .	Intermediate nearer black type	Black type .	Do. .	Intermediate nearer black type
H. Do. .	Do. .	Intermediate nearer green type	Intermediate nearer black type	Black type .	Black type
J. Do. .	Do. .	Intermediate nearer black type	(*a*) Black type . (*b*) Black type .	Almost black type	Do.
L. Do. .	Do. .	Black type .	..	Black type .	..
M. Do. .	Do. .	Intermediate nearer black type	Black type .	Do. .	Black type



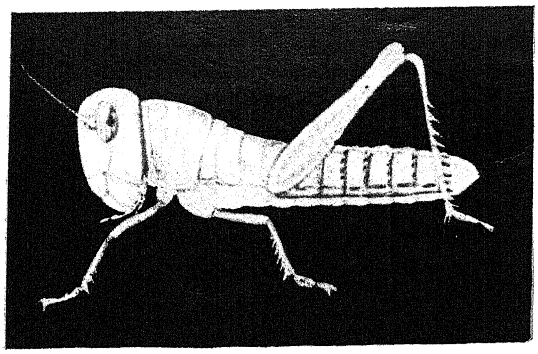


FIG. 1. II stage hopper reared isolated at 44°C. in excess of carbon dioxide.

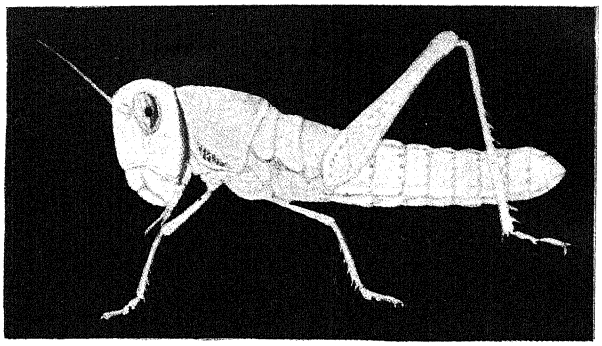


FIG. 2. III stage hopper reared isolated at 44°C. in excess of carbon dioxide.

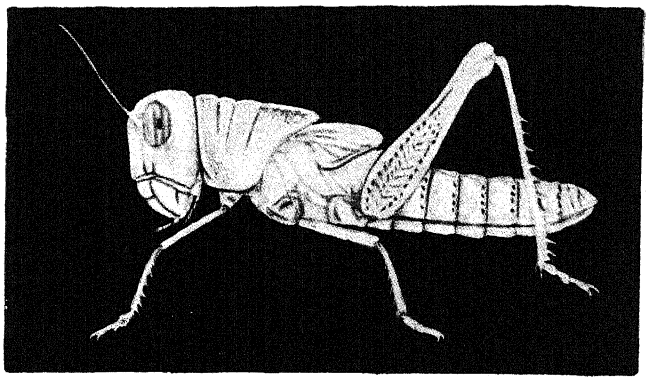


FIG. 3. IV stage hopper reared isolated at 44°C. in excess of carbon dioxide.

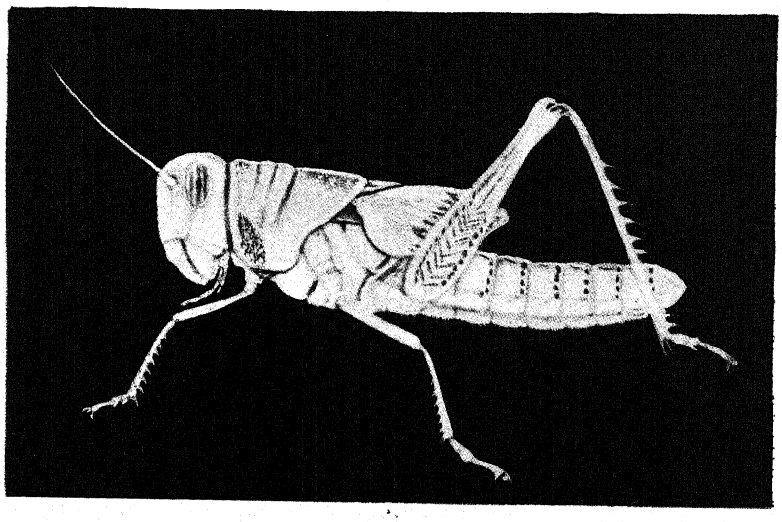


FIG. 4. V stage hopper reared isolated at 44°C. in excess of carbon dioxide.

I. The coloration of a typical V stage hopper reared isolated in atmosphere containing three per cent carbon dioxide at 44°C. (Plate LIX, fig. 4).

(*i*) *General colour.*—Pale yellow all over with scanty deep brown to black pattern.

(*ii*) *Head : Frons.*—Uniformly pale yellow.

Clypeus and Labrum.—Whitish to grey.

Vertex.—Very light brown on both sides of the epicranial suture.

Gena.—Very light yellow. Sub-ocular genal stripe narrow and very light brown.

Post-gena.—Light yellow.

Compound eye.—Very light reddish brown stripes. Inter-stripe yellowish in the anterior half, whitish in the posterior half.

Antenna.—White with a tinge of yellow except the five basal segments which are whitish.

Mandibles, maxillae & labium.—Whitish to grey.

(*iii*) *Cervical sclerite.*—Whitish.

(*iv*) *Thorax : Pronotum.*—Brown patch on the ventral half of the pro- and meso-zonal region of the pronotum. Metazonal triangles deep brown to black but very small. Yellow dots on lighter yellow base all over the pronotum.

Wing-pads.—No black lines on veins or at base of the wing pads.

(*v*) *Legs : I and II pair.*—White.

III pair.—Femur with a narrow pattern consisting of black dots on muscle-impression areas and extending from head of femur to 3/5 its length. Rest of hind leg white.

(*vi*) *Abdomen : I and II segment.*—Uniformly white with a tinge of yellow.

Dorsal streak.—Dark brown and narrow with elongate white triangular areas at posterior margin of each abdominal segment.

Remaining segments.—Four to five small light black dots at the posterior lateral margin of each segment.

J. Experiment VI

History.—Reference No. of parents : P 136.

The solitary adults were received from Pasni on 8th July 1933. In the laboratory the adults were reared in pairs in cages measuring 6-in. × 6-in. × 12-in. The eggs were laid on 2nd August 1933. The hatching consisted of twenty-five green and fifteen intermediate hoppers. Only the green hoppers were selected

for the expriment. The '*solitaria*' tendency was the same as in the hoppers in experiment II. The temperature of the rearing room during the period was :—

Highest maximum . .	35·5°C.	Highest minimum . .	33·5°C.
Lowest maximum . .	30·0°C.	Lowest minimum . .	28·0°C.
Average maximum .	33·9°C.	Average minimum . .	31·8°C.

Average mean temperature—32·85°C.

The aim of this experiment was to determine the maximum concentration of carbon dioxide which *Schistocerca gregaria* hoppers could tolerate.

On 1st August 1932 ten freshly-emerged green hoppers selected from a mixed hatching were isolated and reared in an atmosphere containing about three per cent carbon dioxide. During the first instar two of these died, and the remainder had attained III instar by the 12th September and without exception showed that degree of black pigmentation which is typical of the crowded hoppers at 33°C. At 12 noon on the 12th September the discharge of carbon dioxide into the rearing jar was slightly increased. No marked result was observed till 10 o'clock on 14th September when the carbon dioxide discharge was further increased. At 3 P.M. on the same day six out of the eight hoppers were found dead while the remaining two were in a semi-concious condition and were breathing very fast. A sample of the air at this stage was taken two inches above the bottom of the jar (the position where the two hoppers which were still living were resting on the wire-gauze of the cage) and analysed. This was found to contain seven per cent of carbon dioxide at normal temperature and pressure (by volume). The two hoppers struggling for life were immediately removed from the jar and placed outside. They revived. From 15th September onward they were again subjected to three per cent carbon dioxide atmosphere and they successfully reached the adult stage. Seven per cent of carbon dioxide by volume in an atmosphere, therefore, is a little higher than the maximum concentration in which *Schistocerca gregaria* hoppers can live.

IV. Biometrics

In a number of Locusts it has been shown that besides coloration the difference in their two extreme forms, *i.e.*, phases *solitaria* and *gregaria*, is reflected in the relative size of certain parts. These characters are best expressed, among other things, in the ratio of elytra femur lengths, and, in the ratio of the width of pronotum to its length. As a rule, the ratio of the elytra length to the femur length in a '*solitaria*' adult is lower than that in a '*gregaria*' adult, also the pronotal proportion (width/length) is lower in *solitaria* than in *gregaria* adults.

Since black pigmentation can be acquired by an isolated hopper reared in an atmosphere of excess of carbon dioxide, it is not without interest to determine the influence of this factor on certain morphological characters. For this purpose we restricted our observations to the elytra : femur ratio.

Two sets of experiments were arranged, and the procedure adopted was as follows :—

The hoppers of a single pure green hatching were taken. They were divided into three batches. One batch was reared crowded in a cylindrical cage 8·5 inches high and 4·5 inches in diameter, and fresh leaves were supplied for feeding thrice a day. The hoppers of the other two batches were reared isolated in cylindrical wire-gauze cages six inches high and two inches in diameter. These were also supplied with fresh leaves thrice a day. One of these batches was bred in an ordinary atmosphere, while the other was reared in an atmosphere containing excess of carbon dioxide, which was approximately four per cent.

About twenty-four hours after the final moult the lengths of the right femurs and right elytrae of the adults were measured. The measurements were made by means of a fine pair of dividers and a Vernier scale (metric system). All the hoppers bred crowded and each one of the isolated hoppers bred in an atmosphere containing an excess of carbon dioxide showed patterns of the *black type* hoppers. The isolated hoppers were of the *green type* throughout the hopper stages.

Set I

History.—Reference No. of parents : P 136.

The parents were of the *solitaria* phase and were received from Pasni on 8th July 1933. They were reared as a pair in the laboratory, in a cage measuring 9-in. × 9-in. × 9-in. The date of egg laying was not recorded. The hoppers hatched out on 19th August 1933 and the hatching consisted of seventy-eight hoppers of *green type.*

Conditions.—The experiment was conducted inside the laboratory and the temperature during the period was :—

Highest maximum . .	32·2°C.	Highest minimum .	29·4°C.
Lowest maximum . .	27·7°C.	Lowest minimum . .	26·0°C.
Average maximum .	30·08°C.	Average minimum .	27·09°C.

Food.—Fresh cotton leaves were supplied three times a day.

The measurements of this set are :—

S. No.	Reference No.		Sex	Hind femur length (F)	Elytra length (E)	E:F	Range of E:F
	Isolated in ordinary atmosphere						
1	P136 / 19. VIII. '33	10	Male .	26	52	2·000	1·961—2·083
2	,,	13	Male .	24	50	2·083	

S. No.	Reference No.		Sex	Hind femur length (F)	Elytra length (E)	E : F	Range of E : F
			Isolated in ordinary atmosphere—contd.				
3	P 136 / 19. VIII. '33	25	Male .	25	50	2·000	1·961—2·083
4	,,	7	Male .	24·5	50·5	2·061	
5	,,	9	Male .	26·0	51	1·961	
6	,,	14	Female .	28·4	56	1·975	
7	,,	16	Female .	29	57·8	1·993	
			Isolated in an atmosphere containing 4 per cent carbon dioxide				
1	P136 / 19. VIII. '33	(CO_2B)	Male .	23·5	46	1·957	1·938—2·037
2	,,	(CO_2H)	Male .	25·2	50·2	1·992	
3	,,	(CO_2K)	Male .	24·8	50·1	2·020	
4	,,	(CO_2L)	Male .	27·7	55·3	1·996	
5	,,	(CO_2A)	Female .	27	55	2·037	
6	,,	(CO_2D)	Female .	29	57·2	1·972	
7	,,	(CO_2E)	Female .	28·8	56·7	1·938	
			Crowded in ordinary atmosphere				
1	P136 / 19. VIII. '33	C1	Male .	23	49	2·130	2·092—2·130
2	,,	C4	Male .	24	51·0	2·125	
3	,,	C2	Female .	27	56·5	2·092	
4	,,	C3	Female .	26·8	57·0	2·126	

Set II

History.—Reference No. of parents : L 371.

The parents during their hopper stages were bred isolated and during the adult stage in a pair in a cage measuring 9-in. × 9-in. × 9-in.

The hoppers under experiment hatched out on 29th November 1933, the hatching consisting of sixty-two hoppers of the *green type*.

All the three batches of hoppers were reared in a wooden cabinet which was heated by means of electric lamps and was kept closed. The average temperature during the period was about 32°C. The humidity was high.

Food.—Fresh cotton leaves were supplied thrice a day.

The measurements are :—

S. No.	Reference No.		Sex	Length of hind femur (F)	Elytra length (E)	E : F	Range of E : F
			Isolated in ordinary atmosphere				
1	L371 / 29. IX. '33	33	Male .	25·5	50·8	1·995	
2	,,	14	Male .	26	51·2	1·969	
3	,,	16	Male .	24·8	48·5	1·955	
4	,,	21	Male .	25·0	51·5	2·060	
5	,,	17	Female .	27·5	56	2·040	1·955—2·067
6	,,	8	Female .	29·5	61	2·067	
7	,,	18	Female .	30·2	60·1	1·990	
8	,,	12	Female .	30	59·5	1·983	
9	,,	11	Female .	24·2	48·2	1·991	
			Isolated in an atmosphere containig 4 per cent carbon dioxide				
1	L371 / 29. XI. '33	36	Male .	25·8	49·2	1·907	
2	,,	6	Male .	26·2	53·0	2·023	
3	,,	20	Male .	25	51·1	2·044	
4	,,	26	Female .	28·2	56·0	1·985	
5	,,	2	Female .	26·8	53·5	1·996	1·907—2·098
6	,,	5	Female .	29	61·5	2·120	(except L371 / 29.XI.33 5)
7	,,	23	Female .	28·4	59·5	2·098	
8	,,	19	Female .	27·0	56·5	2·092	
9	,,	30	Female .	27	55·5	2·055	
			Crowded in ordinary atmosphere				
1	L371 / 29. XI. '33	A	Male .	25·2	53·0	2·103	
2	,,	D	Male .	25·8	53·2	2·062	
3	,,	F	Male .	24·6	52·1	2·118	2·062—2·118
4	,,	B	Female .	29·0	60·5	2·091	
5	,,	C	Female .	27·8	Wing damaged		
6	,,	E	Female .	28·4	59·6	2·099	

Table VIII shows the range and the mean value for the ratio E : F, arranged with reference to environment, sex and parentage. It would be seen that the range of the ratios for the isolated hoppers reared in the atmospheres of excess of carbon dioxide falls beyond the range of ratios obtained for the crowded hoppers but lies almost exactly within the range of ratios arrived at in the case of hoppers bred isolated in ordinary atmosphere.

TABLE VIII

Mean E : F with reference to environment, sex and percentage

Environment	Isolated ; atmospheric air		Isolated ; 4 per cent carbon dioxide in air		Crowded : atmospheric air	
Parents . . Sex	P136	L371	P136	L371	P136	L371
Male . .	2·007 (3)	1·994 (4)	1·491 (4)	1·991 (3)	2·125 (2)	2·094 (3)
Female .	1·984 (2)	2·014 (5)	1·982 (3)	2·057 (6)	2·108 (2)	2·095 (2)
Mean . .	1·999 (14)		2·005 (16)		2·105 (9)	
Ranges .	1·961—2·083	1·955—2·067	1·938—2·037	1·907—2·098	2·092—2·130	2·062—2·118

Thus while a hopper reared isolated in an atmosphere containing an excess of carbon dioxide resembles a crowded hopper in the development of black pigmentation, it approaches the hoppers reared isolated, in ordinary atmosphere with regards to its elytra : femur ratio. Further it appears that while in nature the black pigmentation and the higher elytra : femur ratio occur together, these characters may not necessarily always go together. Indirectly our experiment also indicates that whereas the body proportions are dependent on the nature of life lived by the hopper (active or sedentary), the development of black pigmentation may be determined by certain other factors, which may or may not be related to the mode of life of the insect.

V. DISCUSSION

These experiments very clearly show that a green *solitaria* hopper, which, if reared isolated in an ordinary atmosphere, would have retained *solitaria* green coloration, acquired the *gregaria* coloration *i.e.*, black pigmentation, when reared in an atmosphere containing an excess of carbon dioxide. Further the extent and intensity of black pigmentation of these hoppers depends as much on temperature as it does in the case of hoppers bred crowded [Husain and Ahmad, 1936]. Having established this one naturally asks : Is the concentration of carbon dioxide in the atmosphere surrounding the swarming crowded hoppers high enough to induce pigmentation ?

It may be stated that if only two hoppers of the non-swarming type, are reared in a cage measuring 9-in. ×9-in. ×9-in. and covered on five sides with wire gauze of 16 meshes to an inch, they acquire black coloration typical of the swarming type. Under these conditions the percentage of carbon dioxide in the cage cannot be appreciably higher than that in the atmosphere outside the cage. Nor would

the hoppers be always so close together as to inhale any substantial amount of the air exhaled by the other. Therefore the pigmentation in the two hoppers can not be said to have been produced by any excess of carbon dioxide inhaled.

However, it cannot be denied that in a swarm the air immediately around the hoppers will have a higher concentration of carbon dioxide than in an ordinary atmosphere, and also that the air inhaled by the hoppers in a swarm in most cases will contain a large proportion of the carbon dioxide expired by their compatriots. Whilst the carbon dioxide thus inhaled may be playing a significant role in the production of black pigment, it cannot be said that in all cases the excess of carbon dioxide inhaled is the only cause of the development of *gregaria* coloration.

Faure [1932] had advanced a theory, of ' locustine ' production, to explain the development of *gregaria* coloration in swarming hoppers. His explanation is that ' as the result of the excessively high rate of metabolism obtaining in swarm life, the muscles and other tissues produce a surfeit of excretory products that cannot be disposed of by the Malpighian tubules, and these—or their derivatives—are desposited in the cuticula in the form of a definite pattern of black pigment '. These products of excessive metabolism he designates 'locustine '. In the experiments conducted by us the hoppers were isolated in limited space, therefore, the question of the production of a surfeit of excretory products through excessive rate of metabolism that cannot be disposed of by the Malpighian tubules does not arise. It would, however, appear that the production of the *gregaria* coloration is perhaps connected, among other factors, with the respiratory functions of the organism.

It is well known that the hoppers, soon after hatching, manifest coloration characteristic of the phases. In other words they may be black *i.e.*, having coloration of the swarming phase, or green *i.e.*, possess coloration of the *solitary* phase, or they may even be intermediate, *i.e.*, show patterns which are less intense in pigmentation. As a rule the progeny of the *solitaria* parents is green, with no black pigmentation, while that of the swarming parents is black *i.e.*, possesses *gregaria* coloration. Faure explains this phenomenon by saying that " the blood and tissue of the *gregaria* females being more or less saturated with locustine the nutritive yolk of the eggs must also receive it ". Further that " during its development the embryo digests the yolk, and the locustine finds its way into the cuticula to produce the black characteristics of the first instar ". It is well known that quite frequently the hoppers emerging from the same batch of eggs are not all of the same colour, some may be of *green type* while others *black type*. This discriminate distribution of locustine it is difficult to explain.

VI. Summary

1. An excess of carbon dioxide in the atmosphere produces in isolated hoppers black pattern of the type found in swarming hoppers.

2. The black pattern in its intensity and extent is governed by temperature in the same manner as in the crowded hoppers.

3. These hoppers in spite of the production of the black pattern retain in them the *solitaria* body-proportion.

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THE LOCUST INCURSION OF 1935 IN NORTH-WEST INDIA—ITS SIGNIFICANCE IN THE STUDY OF THE LOCUST PROBLEM

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(With two maps and one text-figure)

INTRODUCTORY

During the last locust cycle of 1926-1931, the Desert Locust (*Schistocerca gregaria* Forsk) was active in most of the districts of North-West India either in spring, summer or autumn, and caused a great deal of damage to cultivation. A great mass of data in regard to the details of its activities during this period has been gathered from various sources, chiefly from reports received by the Revenue Departments of the various Provinces and States of North-West India. The material thus accumulated is now under detailed examination. A cursory study of the locust data, however, has shown that in the Indo-Iranian area of infestation, *Schistocerca* breeds in spring in regions subject to winter-rainfall, such as Iran, Baluchistan and parts of the Punjab. The adults of the generation developing from this spring brood are ready to fly between April and June. Beginning from May or June, however, the vast region extending from Dasht-i-Lut and Seistan in Iran to Chagai, Kharan and Panjgur in Baluchistan becomes an area of high temperatures and low humidity with hot, scorching winds blowing from a northern or north-western direction. Locusts produced in the interior of Baluchistan fly out of this area, either south towards the Mekran coast or east towards Sind, Rajputana, Punjab and the United Provinces, their flights being apparently influenced in the latter case by the prevailing south-west winds. As these areas are subject to summer or monsoon rainfall, swarms breed here between June and September, and the summer brood is ready to take to flight by October-November. At this time of the year, the desert areas of Rajputana become a zone of low humidity, which apparently locusts seem to flee from, their flights being directed either

eastwards into the United Provinces and Bengal, or southwards into Central India or the Bombay Presidency, or westwards into Sind, Baluchistan and Iran. These flights are composed of young pink locusts, which may prove very destructive to cultivated crops and to general vegetation. In the case of the westward movements, the swarms show their greatest activity during the months of October and November, when their flights are greatly helped by the prevalent north-east wind. As the cold season advances, the flight movements are greatly retarded, and the swarms usually break up, the individuals getting scattered and passing the winter in a more or less inactive condition in suitable localities. When the weather warms up again with the advent of spring, the over-wintered locusts presumably gather again into swarms. The phenomenon of over-wintering appears to occur wherever swarms happen to be overtaken during their flights by spells of cold weather, and records of the wholesale destruction of swarms in the past in the winter in the uplands of Baluchistan indicate that it is only in the warmer valleys of southern Baluchistan and along the coast that locusts have the greatest chances of surviving the winter.

In the course of a study of the records of locust movements during the last cycle, it has been found that the details of flights are often very meagre, and in certain cases the indicated directions are so confusing that it is difficult to correlate them with other data. On the whole, however, the movements are distinguishable into two principal categories, *viz.*, (1) local movements, in which no unified general direction is discernible, and which are generally associated with a search for forage or for places suitable for egg-laying and (2) mass movements of swarms, associated with a change of the seasons, and characterised by a certain regularity of appearance at particular times of the year and also by a general uniformity of direction. Although it is in general impossible to trace the movements of particular swarms from place to place on account of the imperfections of the recorded reports, it has been actually found possible to follow the progress of a few to a certain extent.

In regard to Sind and Baluchistan, it may be stated that the new swarms of the year always make their entry from a western direction during May, June or July, the first reports being received from the westernmost points, as for example, Bhag and Gandhawa for Kachhi, Dadu, Johi and Shikarpur for Sind, and Jaisalmer and Khairpur areas for the Rajputana desert, and in the course of a few days the further progress of the swarms can usually be traced eastwards. On the other hand, the first swarms of the autumn reach the Baluchistan borders from an eastern direction during October every year.

The locust incursion of 1935

Since December, 1931, locust swarms have ceased to make their periodic appearance in Sind, Baluchistan and Rajputana, and except for a few cases

reported in the cold weather of 1933-34 from the borders of the Rajputana area, no large swarms have been recorded.

Though in the course of surveys made by the Locust staff in the desert areas of Baluchistan and Rajputana, specimens of the non-gregarious, solitary type of locusts had been found in various localities in varying numbers during the last four years, no large concentrations had so far been noted anywhere. The reported appearance, therefore, of a swarm on the 1st July at Shahgarh in Jaisalmer, of another at Reti on the 4th July along the borders of Jaisalmer and Bahawalpore states, and of two other swarms on the 19th and the 29th July respectively in the Khairpore state indicated that, perhaps, a new locust cycle had possibly begun. In addition, the Locust Survey staff had detected a sudden increase of locust population in mid-July in widely separated areas, such as the coastal tracts of Mekran, the Lasbela State, the Kachhi area, the western talukas of Sind, such as Sehwan, Dadu, Johi and Thano Bulakhan, parts of Thar-Parkar and Hyderabad districts, and the desert areas of Jaisalmer, Jodhpur and Bikaner States. At Pasni, where the population rate of locusts on the *rek* areas during the first week of July was only about 500 per sq. mile, the density showed a sudden rise to about 5,000 per sq. mile after the 12th July, and a few weeks later reached about 40,000 per sq. mile. A great many of the locusts noted in July after such increase were, moreover, pinkish in colour, while a certain proportion was bright yellow, and a biometrical examination revealed the existence of *gregaria* ratios in many of them. On the other hand, the comparatively small population existent prior to the 12th July were mostly characterised by the possession of the colour pattern of typical *solitaria* and of ratios of the solitary or intermediate types.

It was evident, therefore, that an incursion of locusts on a large scale had taken place, bringing about the entry of large numbers of specimens of a type approximating the *gregarious* into the Sind-Rajputana area, somewhat in the same manner as if a migration of the usual type in swarm formation had occurred. As the invasion had taken place at a time when the monsoon was beginning to be active in the desert areas, it was feared that a multiplication of the locust in the summer rain areas might inaugurate a new cycle of infestation in the Indo-Iranian area.

The locust incursion of 1935 is remarkable in two ways. First, although in the aggregate a very large number of locust individuals had evidently participated in the incursion, the migration did not take the form of a mass movement of swarms, which one usually associates with a locust invasion. It had, on the other hand, occurred in the form of an imperceptible migration of individuals. This raises the general question of whether the *solitaria* individual is capable of undertaking long distance migrations. As several interesting observations have been made on

this subject during 1935, it is proposed to discuss this subject in greater detail. Secondly, as neither the existence of swarms in Baluchistan nor their entry from outside from neighbouring areas such as Iran had been reported, the migrants of July 1935 could not have originated from swarms. Should this view be correct, their origin could only have been due to a local transformation of the phase *solitaria* into *gregaria*. In other words, local outbreak centres must have come into existence during the year in Baluchistan. As three cases of incipient swarming had come to light during the year, the question will be discussed and its bearing on the subject of control considered.

Migration among the solitaries

The natural habitat of the solitary phase in the Indian area

Although swarms had vanished since December 1931 and locust specimens were not to be seen in most parts of North-West India, solitary specimens were being noticed in greater or smaller numbers in many of the desert areas of Sind, Baluchistan, Rajputana and South-West Punjab by the Locust Survey staff during the years 1932, 1933 and 1934. In many cases, however, the presence of the locust at any particular place was found to be governed by the factor of seasonal distribution. Though, for instance, found in good numbers in the rainy season, they were noticed, with the advent of dry weather to have either completely disappeared or to have become considerably diminished in numbers. On the whole, the preferred habitat of the solitary form of *Schistocerca* was observed to be a sandy area covered with a fair amount of perennial scrub vegetation. Along the Mekran Coast, numerous undulating patches of sand, known locally as '*reks*' are found stretching along the sea-beach, as at Sonmiani, Ormara, Kalmat, Pasni and Gwadar. Originally formed of drifting mounds of sand blown by the sturdy south-west wind from the sea-beach into the interior, they have now become covered over by a scrub vegetation of varying density, which affords both food and shelter to the solitary locust. In the Rajputana area, the vast region of the Indian Desert extending from the shores of the Runn of Cutch for over 600 miles of country up to the borders of the Hissar District, forms another of the favoured habitats of the locust. Being subject to heavier rainfall, the vegetation of the Indian Desert is of a more luxuriant character, and the cover is, on the whole, very much denser, being composed of taller bushes than those on the western '*reks*'. In the desert area the atmosphere is usually drier except during the rains, and the fluctuation of diurnal and annual temperature is greater than in the '*rek*' areas, which being subject to the tempering influence of the sea, are characterised by a fairly humid atmosphere and more equable temperature conditions.

As in the case of the phase *gregaria*, the phase *solitaria* also needs a fairly moist sandy soil for oviposition, and under natural conditions a moist soil would be obtainable only after sufficient rainfall. Consequently the breeding of the solitary phase locust is entirely dependent on the character of the seasonal precipitation.

The western '*reks*' of Mekran fall within the zone of winter rains, the precipitation occurring usually between December and April during the passage of a series of shallow winter disturbances which, starting from the Syrian coast of the Mediterranean Sea, rapidly march eastwards over Iraq, Iran, Baluchistan and Northern India up to the Himalayan Range. On the other hand, the eastern '*reks*' situated in Lasbela State as well as the Indian Desert areas come under the influence of the Indian monsoon, and the rainfall occurs mainly in the summer season between June and September. It, therefore, follows naturally that the locust can breed, in the western areas, only during the winter or spring months usually between February and May, while in the summer rainfall areas, *e.g.*, Lasbela and Rajputana, breeding usually occurs between July and September. During years of drought, breeding may be entirely absent, as had happened at Pasni, Gwadar and Ormara throughout the year 1934, and in the Lasbela area during 1935.

Methods of locust-surveys adopted

During 1931, a rapid preliminary survey of the various parts of Baluchistan was made, chiefly with the object of finding if any permanent breeding areas of the *solitary* phase were existent in that country. As it was found that it was only in Southern Baluchistan that conditions favourable for the breeding and continued existence of the *solitary* locust were existent, a station for studying the life-habits and ecology of the *solitary* phase in its natural breeding grounds was opened at Pasni in January, 1932, and continued observations made during the last four years have proved to be of great value in the investigation of the Locust problem.

One of the points to which particular attention was paid was a study of the fluctuations of the local locust population by conducting periodical surveys in the different areas of the Pasni *reks*. Special attention was paid during the breeding season in regard to making an estimate of the hopper population. The presence of hoppers was detected on the bushes by delicately tapping the branches with a light rod and counting the hoppers found within a particular area. In the case of the adults, it was observed that they do not usually seek the shelter of bushes unless the sand-surface temperature is abnormally high, or the weather too windy, and as a rule, they generally prefer to sit on the surface of open patches of sand. Even in winter, they are so easily started when the sun has warmed up

From the graph, it is evident that both at Pasni and Ambagh, a considerable fluctuation of population had been occurring every year, obviously as the result of more than one factor. First, there is the factor of local breeding as a result of which a rise is noticeable at the close of the breeding season, both at Pasni and Ambagh. At Pasni this is followed, especially in 1933 and 1935, by a rather sudden fall of population, which it would be rather difficult to account for, if our concept be that of a purely local, static population ; for the diminution in numbers is so disproportionately large that it cannot be referred solely to the factor of natural enemies. Contrary to expectation, a distinct rise in population is noticeable at Pasni during May-June 1932, during July-August 1935, and to a slight extent also in May and July 1934, none of these cases being connected with local breeding. Specimens collected at these periods have, moreover, shown fairly high elytron-femur ratios ranging from 2·10 to 2·30. In 1932 and 1935, moreover, the increase of population was definitely known to be due to a general influx of forms of pink, yellow and grey colour from outside.

At Ambagh, an increase in numbers rather out of proportion to the extent and duration of local breeding is noticeable during the months of October and November in 1933 and 1934, and possibly also in 1932. A similar increase is found to have occurred in October and November 1935, in spite of the absence of local multiplication, and in this case, the influx of a recently developed brood of locusts of rather low elytron/femur ratios was definitely established. A similar influx of forms appears to have occurred also at Pasni in October-November 1933, in December 1934 and in November 1935.

In 1935, few locusts are noticeable at Ambagh between January and May, but during June a sudden influx of specimens of a recently developed brood, showing ratios of the solitary phase, was noted. A similar rise in population is noticeable in 1933 and 1934 during the months of May, June and July.

The results of locust-survey work in 1935

During 1935, intensive survey work was in progress in the following places : Gwadar, Pasni and Ambagh in Baluchistan ; at Chachro in Sind ; and at Barmer, Nokh and Sardarshahr in the Rajputana Desert areas. In addition, periodical extensive surveys were also carried out throughout the year in the following parts of (1) *Mekran* : Gwadar-Pishukan-Suntsar ; Dasht-Kulanch ; Kech-Kolwa-Panjgur ; and Kalmat-Ormara ; (2) *Lasbela* : Pohr-Hingol-Uthal ; Hinidan-Shah Bilawal ; (3) *Sind* : Thar-Parkar Desert ; and (4) *Rajputana Desert area* : Mallani-Sheo-Mayajlar ; East Jaisalmer ; Bikaner.

The results of locust findings by the survey staff in all these areas have been marked in the accompanying two maps : Map I, depicting the locust situation during the first seven months from December 1934 to June 1935, and Map II, the situation during the five months—July to November 1935.

Map I : December 1934 to June 1935.—Except for the record of a few locusts noted in June 1935 and of a few stray locusts in Jaisalmer and Bikaner in February-March, locust findings are almost blank for the summer rainfall areas of Lasbela, Sind and Rajputana. On the western *reks*, on the other hand, locusts are seen to be present in abundance. Consequent on the occurrence of heavy winter rainfall, locust breeding was in progress during February, March and April in many places in Mekran both along the coast and in the interior. Adults of the new generation began to appear from the first week of April, and hoppers of the second generation were noted in May-June in Kulanch, Kech and Panjgur.

Map II : July-November 1935.—The situation is vastly different. Very few locusts are seen in the interior of Mekran after July, and the bulk of the locust population is seen to have shifted to the Rajputana desert areas. Early in July, a few swarms were seen in Jaisalmer and Bahawalpore areas, and a few about the middle of July in the Khairpore area. In addition, quite large numbers of locusts including a large proportion of pink and yellow forms appeared in various parts of North-West India : Kachhi, parts of Sind, Marwar, Jaisalmer and Bikaner, and also in the coastal areas of Mekran, in Lasbela and in parts of Karachi District.

In July-August, a fair amount of breeding was noted in various parts of the desert : Thar-Parkar, Mallani and Sheo *parganas* in Jodhpur ; and parts of Jaisalmer and Bikaner. There was no breeding in Mekran and Lasbela. In September-October a few hoppers were still to be found in parts of Thar-Parkar and Mallani, probably representing the second generation of the season.

By November, locusts had in great part disappeared from Bikaner, Jaisalmer and Marwar ; and at the same time, specimens of a new generation of locusts with hyaline or mauve wings were noted appearing in the *reks* of Ambagh, Hingol, Ormara and Pasni.

As already remarked, one of the notable features of the situation during the first half of 1935 was the almost complete absence of locusts in the Lasbela and Sind-Rajputana areas and their presence in the Mekran coastal *reks*. A somewhat similar disappearance was observed in the Thar-Parkar area during the earlier months of the year 1933 and 1934, and their absence was then attributed to the effect of either cold or drought causing them to hide themselves among the vegetation while the cold weather and the drought lasted. In order to test the validity of this assumption, a thorough examination of hundreds of bushes was made during January, February and March in a number of places such as Ambagh, Chachro Derawar, etc. The bushes were uprooted and all likely situations such as their bases and the soil below including the rat-holes were searched, but without finding any locusts in hiding. At Chachro, large thickets of thorn (mostly *Zizyphus* branches) used for fencing fields of cultivation were also lifted up and searched, and though large Acridids like *Anacridium* and *Crytacanthacris* were found taking shelter under them, no specimens of *Schistocerca* were seen. It may, therefore, be taken as proved that the disappearance of the locust from the Ambagh and

Chachro areas during the earlier months of the year was a positive fact, possibly due to seasonal emigration; and consequently the re-appearance of locusts in increasingly large numbers from May onwards at Ambagh and Chachro can only be explained as an immigration from outside.

A biometrical study of locust populations

When a sudden increase in the numbers of locusts was noted at Pasni about the 12th July 1935, a sample collection of the locust population was made and subjected to a cursory biometrical examination, when the existence of a fairly high proportion of forms possessing elytron/femur ratios of the *gregaria* type was detected. Subsequently, an examination of the locust collections on the *reks* at Pasni and elsewhere during the different months of the year 1935 was carried out as far as possible. Later on, all available specimens collected in 1934 were similarly examined biometrically. The results obtained have been tabulated and shown in Appendices I and II respectively. It may be added that in the biometrical studies made, only elytron/femur ratios have been taken into account. No attempts were made to calculate the mean ratios for the different months as the number of specimens available was not sufficiently high.

The results show that the initial population in January on the Pasni *reks* had mostly ratios of the *solitaria* type, and that the progeny of these locusts appearing in April and May also exhibited a dominance of *solitaria* ratios. The July immigrants which swamped the 'native' locust population, however, brought about a preponderance of '*transiens*' ratios with a good proportion of the *gregaria* type. These biometrical characteristics marked the locust population at Pasni till the end of October, even though their numbers had by then greatly diminished. In November, however, an ascendancy of the *solitaria* ratios was again noticeable.

The year 1934 was an abnormal one at Pasni, during which local breeding was completely absent on account of lack of rainfall, and the limited collection of preserved specimens available for examination forms too scanty a material to furnish a basis for any generalisations. Indications, however, are clear that an influx of *gregaria* forms had taken place in May-July, and that an immigration of *solitaria* type had also occurred at the end of the year.

Powers of high flying among the solitaries

When pursued, the *solitaria* adult usually makes only short flights covering not more than a few yards at a time, but its ability sometimes to rise high into the air and vanish out of sight has been noted by the locust survey staff at different times. The significance of this phenomenon was, however, not realised till the event of the July incursion. The fact that the locust population had perceptibly increased by the 12th July and that it included numerous specimens of pink and yellow forms, not known to have been present at Pasni prior to that date, clearly showed that the accretion of population was due to a migration from outside.

Since the arrival of a swarm from outside had not been noticed anywhere round-about Pasni, the inference would appear to be that the migration had been accomplished in the form of individual flights.

After the incursion had taken place, numerous instances of high flying were noted at Pasni. In many instances, locusts were observed to rise high into the air at dusk at an air temperature of 80 to 82° F. (about 27°C.) often without any apparent provocation, and disappear from sight. On some occasions, such high flights were also noted at mid-day. The height attained by the locust could not be accurately estimated, but it must have been at least 500 ft. above the level of the ground. The course of the flight was generally governed by the direction of the prevailing wind, but it was also clear that the locusts did not drift passively with the current, each individual being noted steering its course in a particular way. There is apparently little doubt that the long distance migration of the *solitaria* individual is greatly dependent on the strength and direction of the wind currents prevailing at the time. Since, however, the winds at different levels of the upper air exhibit different directional trends, it is rather difficult to attempt to trace the origin of locust migrants with the help of recorded data on surface currents.

In a few instances, individuals of the locust that were being observed ascending steadily higher and higher into the sky, were found for some unaccountable reason to change their minds, and, after closing their wings, to volplane down to the ground with the speed of a rocket. The swiftness and suddenness of the descent points to the difficulty in noting the actual arrival of the individual migrants, even if it should happen in broad day-light.

Experiments with the marking of locusts

Since February 1935, a system of marking locusts collected during the periodical surveys and liberating them was tried at Pasni with the object of making observations on their powers of movements from place to place. At first, marks indicating the month of liberation were made with cellulose paints on the pronotum of the locusts, but it was found that the paint peeled off after some time. Later on, painting the hind wings was tried, and even in this case there was a similar result, if the paint were applied thick. At present, a system of lightly painting the figure of the month in arabic numerals on the wings is in practice at Pasni. In order to signify the time of liberation more exactly, the left wing is painted during the first fortnight of the month, and the right wing during the second. In addition, a bit of coloured silk thread is tied at the distal end of the femora in order to facilitate their being spotted easily in the field. A similar system is being adopted, as far as possible, at Ambagh and the various Desert Outposts, different colours being adopted by each station : for example blue for Pasni, red for Ambagh, green for Chachro, and so on. The cellulose paints could

not, unfortunately, be obtained early enough for trying the system on an extensive scale during 1935.

Although marking of locusts has been under trial at Pasni since February 1935, it is only in three instances that recoveries have been recorded. (1) A locust painted white in February was captured in March not far off from the place of liberation on the Pasni *rek*. (2) A locust painted red on the hind wing and liberated early in May was found by the Ormara Fieldman at Rumra about twenty miles to the north-east from Pasni on the 17th May. (3) A locust painted blue on the hind wing and let off in June was found within a furlong of the Locust Camp early in July. The second case appears to be very significant.

Liberation was also tried at the following places in 1935 :—

	Month	No. liberated	Results
1. Barmer . .	Sept. .	45 locusts .	None recovered.
	Oct. .	40 locusts .	None recovered.
	Nov. .	15 locusts .	None recovered.
2. Nokh . .	Oct. .	30 locusts .	None recovered.
3. Chachro . .	Sept. .	10 locusts .	None recovered.
	Oct. .	18 locusts .	None recovered
	Nov. .	19 locusts .	Out of specimens liberated during the second fortnight of November, 1935 the following were recovered from the vicinity of Chachro on the dates indicated : 1. on the 5th December 1935 2. on the 30th December, 1935 and 3. on the 8th March, 1936 (in the last case, 3½ months after liberation).

More intensive efforts at the liberation and recovery of marked locusts may be productive of valuable results. Attempts at trying a system of offering rewards for the recovery of marked specimens may perhaps be made, but owing to the sparseness of the population and the difficulty of spotting out the non-gregarious type of the locust by the people, one cannot be over-sanguine about the results.

Summing up of the evidence

Reviewing the results of observations made during the last four years, it may be stated that a considerable volume of evidence has accumulated in regard to the existence among the non-gregarious type of locusts of powers of migration somewhat similar to those exhibited by the *gregaria* forms during years of infestation. Although the data of previous years had vaguely suggested the probability of the occurrence of migration covering short distances on the part of the *solitary* locusts there was no definite evidence in the matter. The results obtained during the year 1935, and especially the observations made at the time of the locust

incursion in July, have served to give fairly clear indications as to the capability of *Schistocerca* to migrate to a distant area by the flight of individuals as contrasted with a migration of swarms. Indeed, although a fairly large increase of locust population had taken place during July in quite a large number of places in Sind, Rajputana and Baluchistan, the fact was not noticed by the local inhabitants in many places, and in all probability the event might have remained unnoticed, had it not been for the activities of the Locust Survey staff stationed in the areas concerned.

The data collected on the subject may be classified under (1) Inductional evidence and (2) Direct evidence.

1. Inductional evidence

(*a*) *Fluctuation of population at Ambagh and Pasni, 1932 to 1935.*—A study of the data collected shows that the fluctuation in the density of population may be quite considerable during a year. If the locust population had been static for each individual breeding ground, the rise and fall in the numbers of locusts should have been capable of correlation with local breeding and climatic conditions. On the other hand, an increase in numbers sometimes occurs prior to the breeding season, and sometimes after the local breeding is over. The details of fluctuation are not explainable except on the basis of the possibility of seasonal migration, leading sometimes to an exchange of population between different breeding grounds and sometimes to an incursion of forms from outside.

(*b*) *The evidence of 1935.*—During the first part of the year, locusts were practically absent from the summer rainfall areas of Lasbela and Rajputana and were concentrated in the winter-rainfall region of Mekran, where considerable breeding and multiplication occurred. On the other hand, during summer and autumn, locusts were to be found in large numbers in Lasbela and Rajputana area, while locusts evidently belonging to a recent brood and possibly hailing from the Indian Desert areas, were found migrating into the western *reks*. These facts indicate that a mutual seasonal exchange of population had occurred between the two areas. The winter and the summer brood areas look as if they were complementary to each other in the scheme of locust economy.

(*c*) *Biometrical evidence.*—Biometrical studies have proved of great value in providing clues for distinguishing between the locust populations prevalent at different parts of the year. They have shown that an original population composed mostly of *solitaria* individuals had been invaded by a large body of migrants exhibiting *transiens* and *gregaria* ratios, in July 1935 at Pasni. By November, this population was found to have gradually disappeared, giving place to forms of a different type, showing mostly *solitaria* ratios and evidently a newly developed brood. Such a replacement of forms at a time when local breeding had not occurred can only be explained by the phenomenon of migration.

2. Direct evidence

(*a*) *Powers of high flying.*—Direct observations have shown that individuals of the *solitary* type are capable of flying high into the air and vanishing from sight. Long distances may, doubtless, be covered in this way by them when backed by favourable winds.

(*b*) *Experiments with marked individuals.*—In the course of experiments with the liberation of marked locusts, it was in one case noted that a marked *solitaria* individual liberated at Pasni early in May 1935 was recovered at Rumra about 20 miles to the north-east on the 17th of May. This is fairly direct proof in regard to the migratory capacity of the *solitary* locusts.

THE OCCURRENCE OF INCIPIENT SWARMING IN MEKRAN

The character of winter rainfall in 1934-35 and of locust breeding

During the year 1934, the winter rainfall proved a failure in most parts of the Mekran Coast, and the only area where good rainfall was recorded was the Jask region in Iranian Mekran where nearly five inches of rain was received in March 1934.

There was no locust breeding noticed anywhere in 1934 within the limits of British Mekran, though doubtless some breeding had occurred in the Jask sector of the Mekran Coast.

The winter rains of 1934-35, on the contrary, proved to be both early and copious all over the winter rain areas of Arabia, Iran and Baluchistan. Rains began in November 1934 in the Bushire and Bahrein areas, and in December 1934 good rainfall was recorded in all the areas, about 1·62 in. at Pasni and 5·25 in. at Gwadar. There were good falls in January in all the Mekran stations. Rainfall continued till the middle of February, after which there was little precipitation along the coast, except at Ormara. Further rainfall occurred, on the contrary, in the interior of Mekran in April, as at Turbat and Panjgur.

With the advent of the winter rains, fair numbers of locusts were found on the coastal *reks* and oviposition probably began early in January 1935. Green hoppers were found in good numbers on all the coastal *reks* during March, April and May. The first adults of the new generation were noted in the first week of April on all the coastal areas. Hoppers were noted also in the interior—in Kulanch and Kolwa during April. By May moisture had disappeared in the *rek* soils along the coast but in the interior valleys of Mekran, wherever further rainfall had been received in April-May, places suitable for oviposition were present, especially along the beds of streams and the banks of the bigger rivers. In Kulanch, fair numbers of hoppers were thus being found during June on bushes of *Chrozophora* sp. along the beds of some of the streams. Possibly, the hoppers of June represented the second generation of the locust.

While the general coloration of the hoppers was found to be green on most of the *rek* areas, a small percentage of brownish green hoppers with darker patches on the thorax and abdomen was also being noted on the Pasni and Ormara *reks* and in Kulanch. Possibly, these may be taken to represent forms of the *transiens* type.

Three reports of gregarious hoppers in Mekran and Kharan

In June and July, reports of the occurrence of *gregarious* hoppers attacking cultivated crops were received from the Kalat State authorities. As they were received very late, a proper investigation of the outbreaks could not be made. The following information was collected by the Fieldman who visited the Mekran areas subsequently.

1. Shashtal area in Kech Valley.—The Turbat Fieldman was informed about June 14th by the Naib of Turbat that a report had been received to the effect that crops at Shashtal near Nodez had been attacked by bands of hoppers. Under instructions from Pasni, he visited the area on the 17th. He was informed by the owner of the field that " about forty days previously bands of black hoppers, hatched on the adjoining sandy *reks*, had entered his field of *jowari* (sorghum) and pulses, and had damaged them. The hoppers had later on turned yellow in colour, and had ultimately disappeared after acquiring wings about ten days ago ". It was reported that various predaceous birds as well as jackals had been attacking the hoppers. The Fieldman reported that he had noted at the time of his visit about 400 adults within an area of 800 sq. yds., as also a number of hoppers of yellow colour on the crops. He also noted the presence of several green hoppers on wild bushes as also of grey coloured adults in the neighbourhood. Locust Research Assistant, Mr. R. N. Batra, visited this area about the 6th July, and could then find only about thirty-three adult locusts in that field.

2. Sehgazan and Gar areas near Panjgur.—On the 22nd July, a report was received from the Naib-Wazir-i-Azam, Mekran, from camp Pasni, to the effect that the Janishin of Panjgur had noticed an infestation by locusts in *jowari* cultivation at Sehgazan on the 7th July and at Gar on the 8th. The area was examined by the Turbat Fieldman on the 12th August, when very few locusts were to be seen. According to his report " the cultivators had encountered about two months ago, a ' flood ' of hoppers of black colour entering the crops at Sehgazan and Gar from the banks of the Rakshan and the Gwargo respectively, and damaging them ". At the time of the visit of the Janishin both hoppers and locusts were noted. All the hoppers had since turned into adults, which disappeared about the middle of July, when the " Gorich " or the dry wind from the north began to blow over the area.

3. Washuk area in Kharan.—The Nawab of Kharan reported to the Political Agent, Kalat about the occurrence of locust hoppers at Balgattar and at Macho in the sandy desert near Washuk at the beginning of July. Information

was later on obtained from the Nawab to the effect that the hoppers had been killed by the intense heat of the desert sands, though perhaps they had in reality become dispersed among the scattered vegetation and been lost sight of.

The significance of these reports

During years of infestation, reports of the occurrence of *gregarious* hoppers would carry no special significance. During the first half of 1935, however, no swarms were reported anywhere, and in the case of the infestations reported from Mekran it was specially stated that no locust swarms had been noted. The appearance of hoppers in large numbers and the assumption of black and yellow coloration by them are rather extraordinary phenomena, not noted since 1932 anywhere in Mekran, and the question as to how these infestations originated is, therefore, of special interest.

In the Shashtal infestation, it is probable that eggs had been laid about the middle of April, while in the Sehgazan and Gar ones, and possibly also in the Washuk case, oviposition had occurred early in May 1935. During the periodical surveys carried out in the Panjgur-Kech-Kolwah areas, locusts were not met with during the months of January, February and March 1935, but were encountered in fairly good numbers in April and May. This circumstance rather suggests that a migration of the solitaries of the coastal areas—made up in part of adults of the new generation and, perhaps, in part of the old generation as well,—had occurred during April, for by that time, the surface moisture of the coastal *reks* had dried up to a great extent making further breeding an impossibility, and the strong south-west wind, which had set in by that time, had possibly helped to carry the migrants into the hinterland.

In the interior of Mekran, which is to a large extent either hilly or stony, places suitable for oviposition are of limited occurrence, being restricted to silt accumulations on the banks of the rivers, mounds of blown silt at the base of hills or the sandy beds of hill streams, and it is possible, therefore, that a concentration of the solitary phase migrants had occurred on these spots at the time of egg-laying, and had led to mass-oviposition on a small scale. The continuance of rainfall in the hinterland in April is a circumstance that must have favoured egg-laying. After hatching, the hoppers had presumably undergone a compulsory crowding in the patches of *jowari* cultivation found in the close neighbourhood, and it is not unlikely that the patches of *jowari* had themselves functioned as the original points of attraction for the *solitary* locusts. In one case, enquiries actually showed that part of the egg-laying had occurred in the field itself.

In all cases, the resultant adults are reported to have disappeared after acquiring wings. As the Fieldman could not examine these infestations early enough, it is not possible to make any statements on the phase characteristics of the new adults, but specimens found by him after the main body had flown away, showed mostly ratios of the *solitaria* type.

These cases are of special importance from the point of view of Locust research. They had occurred not only at a time when there were no swarms about, but also at a juncture just prior to the wide-spread locust incursion noticed in many parts of North-West India in July. If the assumption made as to the origin of these infestations from migrant parents of the *solitary* phase type could be confirmed, these cases would have to be considered to have been actual instances of the transformation of phase *solitaria* into phase *gregaria*, or in other words, the localities where this had occurred should be regarded as some of the actual *outbreak centres*.

Records of outbreak centres in the past

In this connection, it may be recalled [Rao, 1933], that in the year 1923 a locust infestation of a fairly serious character had been recorded at Zahrenbug and Hasadi in cultivated areas in the rich, silt-laden valley of the Dasht River. Hoppers first appeared early in May, and millions of them were being found in a wide area extending from Zahrenbug upto Gabd towards the south. The adults emerging from the hoppers are ultimately said to have disappeared into the jungles by the end of June. It is notable that the infestation had occurred, as in the case of Shashtal, in May-June. There is little doubt that it had originated from migrants derived from the coastal population, which had presumably gone through one generation already by reason of the early rainfall of January 1923. The Kachhi records show that in July-August of the same year, locusts were found damaging *jowari* crops at Dhadhar.

In 1926 also, locusts and locust hoppers were reported to be damaging cotton and *jowari* (*sohru*) crops in the Kulanch area, during the month of June 1926. Locusts were also said to have been present near Tump in June. As there had been very heavy rainfall along the coast and interior of Mekran in January 1926, heavy locust breeding had evidently occurred on the coastal *reks*, and the breeding reported in the silt soils of Kulanch, induced obviously by the good rainfall recorded in the hinterland of Mekran in March and May, presumably represented the second generation of the year. Locusts are reported to have disappeared from Kulanch by the latter half of June, and records show that fliers were in evidence by the end of June in Mirpur-Nasirabad Niabat near Gandhawa in Kachhi and at Kurk near Sibi in the first week of July.

There is little doubt, therefore, that the infestation recorded in 1923 in Dasht Niabat, and in 1926 in Kulanch were of the same character as the Shashtal, Sehgazan and Gar outbreaks, and that they should all be classified as incipient outbreaks produced in the chain of circumstances following early and plentiful winter rainfall in the Mekran areas.

The probable origin of the July incursion

The instances of incipient swarming reported this year from Mekran show that wherever conditions are favourable for development, a second generation

of the locust would be produced. As Mekran is a vast area with a very sparse population, it is not unlikely that besides the cases reported, similar other outbreak centres had come into existence during the year, but had remained unreported as the damage was not considered serious enough. Similarly, it should not be considered surprising if such swarming had also developed unnoticed in uncultivated areas supporting sufficient vegetation. In any case, there is little doubt that there were large numbers of locusts present in various parts of Mekran during June and July. Since in the adjoining province of Iranian Baluchistan, conditions were presumably identical, it is not improbable that similar outbreak centres had come into existence at about the same period, and if so, a considerable multiplication of the locust population must have taken place in that area also.

From May onwards, the climatic conditions of the hinterland of Mekran usually undergo a gradual change from the temperate character of spring to the extremely torrid conditions of summer, characterised by high temperatures and low humidity and the prevalence of dry hot winds from the north or north-west, so that by June or July a zone of dry and hot climatic conditions develops in an extensive area inclusive of large parts of Persia and Afghanistan, which locusts are obviously unable to stand and try to flee from. The local wind directions would carry them either southwards to the coastal areas or eastwards into the plains of India. When locusts enter the monsoon zone, the prevalent south-west wind would carry them into the areas of Kachhi, Sind and Rajputana. Lean's observations [Lean, 1931] in Nigeria show that the swarms of *Locusta migratoria migratorioides* prefer a zone of relative atmospheric humidity between the limits of eighty and forty per cent. and try to avoid regions above or below those limits. *Schistocerca* apparently exhibits a similar dislike for very dry as well as very humid atmospheric conditions. Ballard and Mistikawy have shown [Ballard and others, 1932] that seasonal winds have a great influence on the directional trends of swarms of the Desert Locust. What applies to swarms may also apply equally to individual flights.

In view of the facts detailed above, there appears to be little doubt that the locust incursion of July 1935 had had its origin in part at least in the Mekran area, to which the centres of swarming noted at Shashtal, Sehgazan, Gar and Washuk had apparently contributed their quota, though, on account of the extraordinarily high numbers characterising the incursion, it is probable that the bulk of it must have had an extra-Indian origin.

Breeding in the summer-brood areas

Although fairly large concentrations of locusts had been noted in July in quite a large number of places in Sind and Rajputana, breeding was greatly restricted on account of the lack of well-distributed rainfall. There was no rainfall, and consequently no breeding whatever, in Lasbela and Karachi areas and in

Mekran. Good rainfall occurred only in the Bikaner area, in South Marwar and Thar-Parkar, and even in these regions there were only light falls in August and September.

Comparative rainfall figures in 1935 and 1926 in inches

Places	1935			1926		
	July	August	Sept.	July	August	Sept.
Barmer . .	4·34	0·80	1·50	3·51	4·17	8·35
Chachro . .	8·18	2·47	0·49	2·25	7·91	5·52

A fair amount of breeding was noted in Thar-Parkar, Mallani and parts of Bikaner during July-August, but on account of the insufficiency of rainfall, breeding was neither dense nor extensive. On the other hand, owing to the heavier and continued monsoon in 1926, the breeding was so heavy as to start a new cycle of locust infestation.

The first adults of the new generation were noted at the end of August 1935. Some hoppers were still being found in October and November in Thar-Parkar District, which probably represented the second generation of the monsoon. After October the locust population in the Rajputana desert began to show a gradual decrease in numbers, till by the end of November very few could be seen except in the Thar-Parkar area. Since about the same time, the arrival of solitary specimens of the new generation was detected in October-November in the Lasbela area, and at Pasni in November, a migration from east to west had obviously occurred.

A cursory study of the meteorological data has shown that, with the withdrawal of the monsoon in September, a zone of low humidity develops in the Rajputana area, which apparently causes the locusts to migrate out of the area. At the same time a change in the direction of the prevailing wind from south-west to north-east, taking place about the middle of October, presumably plays a great part in bringing about the westward migration of the locusts from the desert area.

Conclusions

In reviewing the various observations made in 1935 in connection with the locust developments of the year, it may be stated that they have led to a considerable advance in our knowledge of the life-habits of the Desert Locust. Some deductions that may perhaps be made in regard to the mode of origin of the infestation in the Indian area are briefly outlined below, and a few notes have also been added in connection with the aspect of control in the breeding grounds.

1. It appears probable that early and copious winter rainfall would lead to extensive breeding on the coastal *rek* areas and bring about a great increase in the general population. The new generation of adults would appear by April and migrate into the interior in the absence of conditions favourable for further breeding on the *reks*. Should there be rainfall at this juncture in the interior of Mekran, concentrated egg-laying would occur in suitable areas, leading to incipient swarming.

2. The adults of the second generation would migrate into Sind and Rajputana by individual flights, or perhaps as small loose swarms, and if the monsoon rainfall should be favourable, as in 1926, further multiplication would occur and lead perhaps to the formation of large swarms. Otherwise the locust invasion would fizzle out.

3. The flights that are responsible for the starting of a new cycle of infestation in India would appear always to come from the direction of Mekran, but this is not generally recognised, as the arrival of locusts may take the form of a migration of individual locusts as during 1935, and is then not easily noticed. This had presumably happened in 1926, and probably also in 1923. In both these years, the entrance of locusts in June-July into India is indicated by records of actual occurrence of locusts at this period in parts of the Kachhi area in July 1926 and in July-August 1923 on cultivated crops. In studying the origin of locust cycles of the past, prior to 1926, in India, due consideration should be given to this circumstance owing to the general incompleteness of the records.

4. The evidence found in regard to the capability of the solitary locust for migration indicates that it is eminently adapted to escape the effects of local drought. If winter rains should fail in one area, it could always migrate to another better favoured area, and, in the last resort, may be able to reach regions of summer rainfall. Usually there appears to be a mutual exchange of population between the western *reks* and the desert areas, and since they function equally well as regions of habitat of the locust, they should be considered to be complementary to each other.

5. In devising measures to check the swarming of the locust, it would appear futile to attempt to deal with the general breeding in the areas of habitat, as on account of the extent of the area and the scattered nature of distribution of the *solitary* locusts control measures would be uneconomical. On the other hand, it would be comparatively easy to deal with the concentrations of hoppers appearing at the time of incipient swarming. Special attention will, therefore, have to be paid to the location of such outbreak centres and prompt steps will have to be taken to deal with the incipient swarms. Since outbreak centres of the Indo-Iranian region are likely to develop in Iranian Mekran also, international co-operation would be essential, to ensure efficiency of control.

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EXPLANATIONS OF FIGURE AND MAPS.

Fig. 1.—Graph showing the fluctuation in the locust population in the Pasni and Ambagh *Reks* during a period of four years, 1932 to 1935.

Maps.—Map I : showing the distribution of the Locust in the breeding grounds in the Indian area between December 1934 and June 1935.

Map II : showing the distribution between July and November 1935.

APPENDIX I

Results of a biometrical analysis, month by month, of a series of locust specimens collected mainly from the Pasni reks during 1935

N.B.—For purposes of convenience, the following E/F ratios will be considered to signify the different phases :
2·05 and below : *Solitaria.*
2·06 to 2·15 : *Transiens* or *Intermediate.*
2·16 and above : *Gregaria.*

Month	Place of collection	Total number of specimens examined	Phase of the specimens	Total number of each phase	E/F ratios	Remarks
January, 1935	Pasni	12	Sol.	11	1·97, 1·99, 2·00, 2·00, 2·01, 2·02, 2·03, 2·03, 2·04, 2·04, 2·04.	Mostly *solitaria* and with traces of purple in hind wings.
			Int.	1	2·15.	
February, 1935	Pasni	10	Sol.	9	1·98, 2·00, 2·00, 2·02, 2·04, 2·04, 2·05, 2·05, 2·05.	Veins with traces of purple in hind wings, which are mostly yellow.
			Int.	1	2·07.	
March, 1935	Pasni	7	Sol.	5	1·93, 1·95, 2·03, 2·03, 2·05	Hind wings yellow.
			Int.	2	2·06, 2·13.	
April, 1935	Pasni	4	Sol.	3	2·04, 2·05, 2·05	Some with hyaline wings.
	Kalmat		Int.	1	2·09 (Hyaline wings).	
May, 1935	Pasni	8	Sol.	6	1·93, 2·00, 2·03, 2·03, 2·03, 2·05	Some with hyaline wings, others with bright yellow ones.
	Ormara		Int.	2	2·07, 2·08.	
	Kulanch					
June, 1935	Non-available.					
July, 1935	Pasni	19	Sol.	12	1·94, 1·95, 1·98, 2·00, 2·01, 2·03, 2·04, 2·04, 2·04, 2·04, 2·04, 2·05.	Collected mostly between the 12th and 15th July.
			Int.	4	2·09, 2·12, 2·13, 2·15.	An incursion of locusts on a large scale took place about the 12th July and the migrants were largely *transiens* and *gregaria.*
			Greg.	3	2·16, 2·18, 2·24.	
	Ormara	1	Greg.	1	2·29.	
August, 1935	Pasni	21	Sol.	5	1·94, 2·01, 2·01, 2·03, 2·03.	Population composed largely of migrants.
			Int.	10	2·06, 2·07, 2·07, 2·09, 2·10, 2·13, 2·13, 2·13, 2·15, 2·15.	
			Greg.	6	2·16, 2·19, 2·19, 2·21, 2·22, 2·23.	
	Ormara area	60	Sol.	9	1·90, 1·96, 1·96, 1·98, 2·00, 2·02, 2·02, 2·03, 2·05.	Many of the specimens were either pinkish or had a pinkish tinge in the hind wings ; some were bright yellow, especially the males. Others had fairly conspicuous stripe
			Int.	38	2·06, 2·06, 2·07, 2·07, 2·07, 2·08, 2·08, 2·09, 2·09, 2·09, 2·09, 2·09, 2·10, 2·11, 2·11, 2·11, 2·11, 2·11, 2·11, 2·12, 2·12, 2·12, 2·13, 2·13, 2·13, 2·13, 2·13, 2·14, 2·14, 2·14, 2·14, 2·14, 2·14, 2·15, 2·15, 2·15, 2·15, 2·15, 2·15.	
			Greg.	13	2·17, 2·19, 2·19, 2·20, 2·21, 2·22, 2·22, 2·22, 2·23, 2·25, 2·25, 2·29, 2·34.	

APPENDIX I—*contd.*

Month	Place of collection	Total number of specimens examined	Phase of the specimens	Total number of each phase	E/F ratios	Remarks
September, 1935	Pasni 31 Ormara 2	33	Sol.	8	1·95, 1·96, 2·00, 2·00, 2·01, 2·04, 2·05, 2·00.	Mostly composed of July migrants, but the general population had decreased; almost all of the specimens met with showed conspicuous stripes of the *Solitaria* type, though the ratios varied a great deal. Most had yellowish wings.
			Int.	16	2·06, 2·07, 2·07, 2·07, 2·09, 2·09, 2·09, 2·10, 2·10, 2·10, 2·11, 2·13, 2·14, 2·14, 2·15, 2·12.	
			Greg.	9	2·16, 2·18, 2·19, 2·20, 2·21, 2·21, 2·22, 2·24, 2·26.	
October, 1935—						
12-17/-X-35	Pasni	15	Sol.	4	2·00, 2·01, 20·2, 2·05	Population had greatly diminished, specimens rather dark and conspicuously striped. Hind wings showed traces of mauve and light blue. Mostly remnants of the July migrants.
			Int.	5	2·06, 2·07, 2·13, 2·14, 2·15.	
			Greg.	6	2·16, 2·16, 2·17, 2·17, 2·19, 2·23.	
28-30/-X-35	Pasni	21	Sol.	8	1·94, 1·94, 1·96, 1·96, 1·96, 1·97, 2·03, 2·04.	
			Int.	6	2·06, 2·08, 2·09, 2·10, 2·11, 2·15.	
			Greg.	7	2·16, 2·16, 2·16, 2·17, 2·17, 2·18, 2·21.	
November, 1935— 1st fortnight	Pasni	24	Sol.	14	1·94, 1·95, 1·96, 1·97, 1·97, 1·98, 1·98, 1·99, 2·01, 2·01, 2·03, 2·03, 2·04, 2·04.	The proportion of *Solitaria* greatly increased during the month, and as most of them had bluish or purplish hyaline hind wings, they probably belong to a recent brood. As similar forms have been noted at Ambagh, Hingol, and Ormara since the middle of October, it is considered that they are probably migrants from the Rajputana Desert areas. A few of the old July migrants also found.
			Int.	10	2·06, 2·08, 2·08, 2·11, 2·11, 2·13, 2·13, 2·14, 2·15, 2·15.	
2nd fortnight	Pasni	27	Sol.	20	1·83, 1·92, 1·92, 1·93, 1·93, 1·95, 1·95, 1·96, 1·97, 1·97, 1·98, 1·98, 1·99, 2·00, 2·00, 2·00, 2·00, 2·00, 2·04, 2·05.	
			Int.	5	2·10, 2·11, 2·11, 2·12, 2·14.	
			Greg.	2	2·16, 2·22.	

APPENDIX II

Results of a biometrical analysis, month by month, of all available locust specimens collected mainly from the Mekran Coastal reks during 1934

N. B.—For purposes of convenience, the following E/F ratios will be considered to signify the different phases :
2·05 and below : *Solitaria.*
2·06 to 2·15 : *Transiens* or *Intermediate.*
2·16 and above : *Gregaria.*

Month	Place of collection	Total number of specimens examined.	Phase of the specimens	Total number of each phase	E/F Ratios	Remarks
January February, and March, 1934	Preserved specimens not available.					
April, 1934	Gwadar 1 (G) Pasni 3 (P) Ormara 3 (O) Awaran 1 (A)	8	Sol. Int.	6 2	1·90 (O), 1·92 (A), 1·95 (O), 2·00 (O), 2·00 (P), 2·03 (P). 2·07 (G), 2·13 (P).	Specimens mostly with bright stripes and yellow hind wings.
May, 1934	Pasni 3 Gwadar 1 (G)	4	Sol. Int. Greg.	1 2 1	1·92 2·07, 2·12. 2·20 (G).	2·20 with almost hyaline, and 2·12 with pinkish hind wings. Apparently both were recently fledged ones.
June, 1934	Pasni	2	Sol.	2	1·98, 2·01.	
July, 1934	Pasni 2 (P) Pishukan 1 (Pn) Kappar 1 (K)	4	Sol. Int. Greg.	*Nil* 2 2	..... 2·10 (K), 2·11 (P). 2·20 (Pn), 2·26 (P).	All specimens appear to be locusts of a recently developed brood.
August, 1934	Pasni 1 (P) Gwadar 1 (G)	2	Sol. Int.	1 1	2·03 (G) 2·06 (P).	Locusts scarce in all *reks.*
September, 1934	No specimens preserved.					
October, 1934	Gwadar	2	Sol.	2	2·00, 2·04	2·04 Hind wings purplish; stray specimens found at Gwadar, but none on the Pasni *reks.*
November, 1934	...	...	...	...	.....	No locusts were found anywhere on the Mekran *reks.*
December, 1934	Gwadar 1 (G) Pasni 6	7	Sol. Int.	5 2	1·93, 1·96, 1·96, 2·01, 2·01 (G) 2·06, 2·07.	All specimens showed purplish tinge in the hind wings. They appear also to be a recently developed brood.

SINGLE-VALUE SOIL PROPERTIES : MOISTURE AT THE STICKY POINT AND *R*

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CONTENTS

	Page
I. Introduction	1054
II. The soils used	1055
III. Methods employed	1055
IV. Experimental results—	1057
(*a*) Effect of treatments on mean values of carbonates, organic carbon, etc.	1057
(*b*) SP values	1058
(*c*) R values	1059
V. Discussion of results	1060
VI. Conclusions and summary	1062
References	1063
Appendix	1063

I. Introduction

Working with soils of the same type from a restricted area, Prescott and Poole [1934] have shown that mechanical analysis may be related to certain single values such as Moisture at the Sticky Point, Moisture Equivalent, etc., in a simple fashion. That different replaceable bases may have an influence on single value soil properties has been shown by Baver [1928] and by the writer [Charlton, 1932]. Baver's results were not available to the writer until quoted in a paper by Baver and Hörner [1933]. In the case of Lower Burma paddy soils, correlations as high as $r=0\cdot93$ had been found between mechanical analysis expressed as clay and silt and the moisture at the Sticky Point and $r=0\cdot95$ between mechanical analysis and the value *R*. Such high values were only obtained after hydrogen peroxide treatment and saturation with a single cation, preferably H′ or Ca″. The soils used were those for which single values have already been published [Charlton, 1932]. Although the regression equations connecting moisture at the Sticky Point and *R* with mechanical analysis enabled the values to be calculated with considerable accuracy, the equations were not applicable to the

calcareous soils of Upper Burma even after treatment with hydrogen peroxide and saturation with the same cation as was used with the Lower Burma paddy soils. The object of the work described in this paper was to discover the reasons underlying this difference in behaviour.

II. The soils used

Thirty-eight soils were used. Of these Hmawbi, B. 610, B. 616, B. 618 and B. 624 are Lower Burma paddy soils. These soils are all fairly highly leached soils on old alluvium. B. 951, B. 957, B. 988, B. 992, B. 999, B. 1010, B. 1015 and B. 1031 are typical tropical or sub-tropical red soils at an elevation of about 2,000 feet under a rainfall sufficient to remove all but small amounts of replaceable bases. These soils are under forest planted to *Gmelina arborea*, a tree requiring good drainage for successful growth. Soils B. 1129, B. 1131, B. 1133 and B. 1135 are sugarcane soils in the Bilin tract, subject to occasional flood and are under an annual rainfall of 200 in. per annum or more. B. 1137, B. 1139, B. 1141 and B. 1143 are sugarcane soils from Shwegun also under a rainfall of at least 200 in. per annum but are not subject to flood. Nanda Tank and Mandalay soils Nos. 1-57 are calcareous Mandalay-Canal area soils which in their natural condition without irrigation approach closely the Solontschak type, while the three Singaing soils differ little from the Mandalay type but being closer to their canal headworks tend to receive more silt in the irrigation water and being rather lighter in texture than the usual Mandalay-Canal area soils grow sugarcane as well as paddy. The thirty-eight soils so chosen were deliberately selected to include wide variations in geological formation, rainfall and texture. It will be noted that although none of the soils is a laterite a few are lateritic according to Martin and Doyne's definition [1927] while the ratio SiO_2/Al_2O_3 (molecular) covers the range 1·62-4·30.

III. Methods employed

(*a*) *Clay Equivalent* (CE).—The method of mechanical analysis used was the revised British Official Method [1928] with the exception that organic matter was removed by Troell's method [1931] using cold NaBrO solution instead of hydrogen peroxide. This method was found preferable to hydrogen peroxide since sesquioxides were not dissolved and further, whereas hydrogen peroxide was found to be practically without effect on the organic matter of calcareous soils, oxidation by cold NaBrO solution proceeded smoothly and as efficiently as in sour or neutral soils. In certain soils containing large amounts of organic matter two treatments were necessary. Russell [1933] has shown that more than eighty per cent of the total variance of the Xylene Equivalent and Moisture Equivalent is accounted for by the clay and fine silt fractions and that the residual variance is not decreased by taking into account the fractions coarser than fine silt. Hence, since in the Atterberg scale the silt fraction is ten times the size of the clay it was considered justifiable on the grounds of simplification

to add one-tenth of the silt fraction to that of the clay and to utilize the value so found as a single term called the " Clay Equivalent ". Certain unjustifiable assumptions are thereby implied but the mathematical treatment is so much simplified that it was considered justifiable to make use of the conception.

(*b*) *Base Exchange Capacity* (BEC).—The method of F. W. Parker [1929] using his barium acetate-ammonium chloride procedure was employed.

(*c*) *Moisture retained by soil in 50 per cent saturated atmosphere* (R).—Thin films of soil, each of approximately equal weight were exposed in identical squat form weighing bottles to sulphuric acid of appropriate strength at 30°C.

(*d*) *Moisture at the Sticky Point* (S).—The method of Keen and Coutts [1928] was followed with the modification that all soils were first of all made rather too moist and were then worked in the fingers until sufficient moisture had been lost to reduce the soils to the desired condition.

(*e*) *Preparation of calcium soils.*—Two series of calcium soils were prepared. In one series the organic matter was removed by treatment with cold NaBrO solution while in the other this treatment was omitted. After treatment with NaBrO in the first series, excess of bromine was removed by dilute ammonia and then the soils were treated twelve times by decantation with *M* calcium chloride solution, transferred to filter papers, washed twelve times with *M*/10 calcuim chloride solution and were finally washed with freshly boiled distilled water until the filtrates were free from calcium and chlorine ions. In the second series the treatment was identical except that the NaBrO treatment was omitted, the soils being directly treated with *M* calcium chloride solution.

(*f*) *Silica-sesquioxide ratio* (*molecular*).—The clay fractions of the soils were separated after removal of organic matter by cold NaBrO and silica and sesquioxides in the clay were then determined after fusion of the clay with excess sodium carbonate.

Carbon dioxide, loss on ignition, etc. were determined in the usual way. In the case of the untreated (original) soils, no treatment with ammonium carbonate was given in determining loss on ignition as such treatment is likely to affect the results to some extent. With calcium chloride and NaBrO treated calcium soils, after ignition the soils were ' moistened ' with ammonium carbonate solution and dried to constant weight at 105°C. Organic carbon was determined by wet combustion and the values so obtained were multiplied by the conventional factor 1·818 to give the organic matter. The difference between loss on ignition and organic matter was assumed to be the combined water (*CW*).

It has been assumed in this paper that a single set of values for *CE*, *BEC* and $\frac{SiO_2}{R_2O_3}$ is applicable to all soils whether untreated, treated with *M* calcium chloride only or with NaBrO solution followed by *M* calcium chloride solution. In the case of *CE* the point is not capable of direct proof since in mechanical analysis the

clay is usually determined as ammonium or sodium clay and not as calcium clay. The fact that the *BEC* did not alter significantly as a result of either calcium chloride or NaBrO followed by calcium chloride treatment was proved by actual test on twelve of the soils. In the case of $\frac{SiO_2}{R_2O_3}$ the fact that no loss of silica and sesquioxides could be detected as a result of NaBrO treatment was taken to justify the procedure.

IV. Experimental results

The data obtained for original, calcium and NaBrO treated calcium soils are detailed in Appendix Tables I-III. The total and fifth order partial correlations are given in Appendix Tables IV-VI, all partial correlations except the fifth order being omitted in order to economise space. The level of significance ($P = 0{\cdot}05$) is about $r = 0{\cdot}35$ for a fifth order and $r = 0{\cdot}325$ for a total correlation. In a few cases partial correlations of a lower value than $r = 0{\cdot}35$ have been provisionally used in formulating the regression equations between *SP* and *R* in terms of the parameters *CE*, organic carbon, *CW*, etc.

(*a*) *Effects of treatments on mean values of carbon dioxide, organic carbon, etc.*

The effects of the various treatments employed may first be considered. Results are shown in Table I.

Table I

Effect of treatments on mean values

	CO_2 as carbonate	Organic carbon	Combined water	*SP* moisture	*R*
Untreated soils . . .	1·604	1·398	5·074	40·85	3·361
Calcium soils . . .	1·770	1·489	4·013	41·70	3·393
NaBrO treated calcium soils	1·944	0·828	3·737	40·10	3·104

In considering these results it should be remembered that certain of the determinations are calculated on air-dry soil and as the amount of moisture in air-dry soil may vary as a result of treatments employed, small differences may not be significant. Also certain parameters are expressed on oven-dry soil. There can be no doubt, however, that NaBrO solution removes about forty per cent of the organic matter on the average but the various treatments do not have a very pronounced action on mean values of *SP* and *R*. The most noteworthy effect of the treatments is on *CW*, a reduction of about twenty per cent being effected by simple treatment with calcium chloride solution. Further, this reduction in *CW* is a mean result for all soils, half of which are calcareous. If the Mandalay and Sagaing soils are excluded the reduction of *CW* as a result of calcium chloride treatment (without NaBrO) is 34·7 per cent. This reduction in *CW* (and hence

of *L 1*) as a result of replacement of H′ by Ca″ is the most important fact upon which the equations for *SP* and *R* are derived. If the difference between mean results for calcium and NaBrO treated calcium soils may be attributed solely to removal of humified organic matter it appears that unit weight of such organic matter may take up 2·42 times its own weight of water as *SP* and 0·443 times its own weight as *R*.

(*b*) SP *values*

The fifth order partial correlations detailed in appendix Tables IV-A, IV-B and IV-C show that *SP* is controlled primarily by *CW* and to a less extent by organic carbon and $\frac{SiO_2}{R_2O_3}$ the partial correlations between *SP* and organic carbon and $\frac{SiO_2}{R_2O_3}$ being slightly less than significant in certain cases. As would be expected, the untreated soils show quite different relationships from the calcium and NaBrO treated calcium soils and not much importance can be attached to the values for untreated soils. It was expected that the values obtained for calcium and NaBrO treated calcium soils would check each other fairly closely except as regards the behaviour of organic matter which is of quite different nature in the two cases and of course thereby affects the values for *CW*. Actually the fifth order partial correlations in the cases of calcium and NaBrO treated calcium soils check sufficiently well to support the conclusion that in such soils (saturated with one cation only) *SP* is controlled primarily by *CW* and secondarily by organic carbon and $\frac{SiO_2}{R_2O_3}$. The substitution of the single value loss on ignition (*L 1*) for the two values *CW* and organic carbon in the same soil causes no noticeable reduction in the value of the multiple correlation coefficient and so allows of further simplification. The actual regression equations are given in Table II.

TABLE II

Equations for SP

	Multiple correlation coefficient
A (1) *Original soils*	
$SP = 0{\cdot}1294\,CE + 4{\cdot}929$ org. C $+ 1{\cdot}591\,CW - 0{\cdot}3143\,BEC + 2{\cdot}245\frac{SiO_2}{R_2O_3} + 19{\cdot}98$	0·8490
A (2) *Calcium soils*	
(a) $SP = 4{\cdot}456$ org. C $+ 2{\cdot}766\,CW + 3{\cdot}206\frac{SiO_2}{R_2O_3} + 16{\cdot}78$	0·8826
or	
(b) $SP = 2{\cdot}637\,L1 + 3{\cdot}221\frac{SiO_2}{R_2O_3} + 16{\cdot}76$ . . .	0·8838
A (3) *NaBrO treated calcium soils*	
(a) $SP = 5{\cdot}718$ org. C $+ 4{\cdot}040\,CW + 5{\cdot}800\frac{SiO_2}{R_2O_3} + 7{\cdot}28$.	0·9028
or	
(b) $SP = 3{\cdot}708\,L\,1 + 6{\cdot}267\frac{SiO_2}{R_2O_3} + 6{\cdot}62$	0·9015

The increase in value of the multiple correlation coefficient as a result of the treatments given is not significant but this may be attributed to the greater number of constants fitted in the case of the untreated soils. Apart from the fact that substitution of *L 1* for *CW* and organic carbon causes practically no change in the multiple correlation coefficient, the calculated values for *SP* detailed in Appendix Table VII-A are practically identical. It may be noted that in the case of the calcium soils, the constant approaches closely to 16, *i.e.*, the usual limiting value for very sandy soils in closest packing. In the case of NaBrO treated calcium soils the constant has a much smaller value and hence it appears likely that NaBrO has a pronounced effect on the packing of the soil particles.

(*c*) R *values*

The fifth order partial correlations detailed in appendix Tables IV-B and IV-C do not check so well as in the corresponding partial correlations for *SP*. As in the case of *SP* however, *CW* is the most important parameter while *BEC* gives a significant correlation in all cases. *CE* is on the verge of significance for NaBrO treated soils only. Carbon dioxide is significant in the case of original and calcium soils only while $\frac{SiO_2}{R_2O_3}$ is significant in the case of original and calcium soils only. The omission of $\frac{SiO_2}{R_2O_3}$ in the case of NaBrO treated calcium soils causes the calculated values given in Appendix Table VII-B to fall in to classes determined by soil type, in spite of the comparatively low partial correlation coefficient ($r = 0{\cdot}1949$) between R and $\frac{SiO_2}{R_2O_3}$. In the case of NaBrO treated calcium soils, two equations have therefore been given, one with and one without taking into consideration $\frac{SiO_2}{R_2O_3}$.

The equations for R are given in Table III.

TABLE III

Equations for R

	Multiple correlation coefficient R
B (1) *Original soils.*	
$R = 0{\cdot}1819\ CO_2 + 0{\cdot}2139\ CW + 0{\cdot}1437\ BEC + 1{\cdot}325\ \frac{SiO_2}{R_2O_3} - 3{\cdot}129$	0·9166
B (2) *Calcium soils*	
$R = 0{\cdot}1053\ CO_2 + 0{\cdot}3828\ CW + 0{\cdot}1428\ BEC + 1{\cdot}002\ \frac{SiO_2}{R_2O_3} - 2{\cdot}708$	0·9572
B (3) *NaBrO treated calcium soils*	
(a) $R = 0{\cdot}03121\ CE + 0{\cdot}4030\ CW + 0{\cdot}06065\ BEC - 0{\cdot}653$	0·9126
or	
(b) $R = 0{\cdot}01111\ CE + 0{\cdot}4918\ CW + 0{\cdot}0870\ BEC + 0{\cdot}7828\ \frac{SiO_2}{R_2O_3} - 2{\cdot}266$	0·9371

The improved values of the multiple correlation coefficient as a result of soil treatments are not significantly better than that for the untreated soil. Even when $\frac{SiO_2}{R_2O_3}$ is included in equation B (3) (b) the soils still tend to fall into classes according to their origin but the agreement between observed and calculated values is better for equation 3 (B) (b) than for 3 (B) (a). The constant becomes relatively large when $\frac{SiO_2}{R_2O_3}$ appears because the minimum value of $\frac{SiO_2}{R_2O_3}$ for any soil is 1·42 and the average 2·24. It appears that NaBrO treatment has proved unexpectedly drastic in its effect on *R*. The partial correlations for carbon dioxide, *CW*, *BEC*, $\frac{SiO_2}{R_2O_3}$ and *R* check very well in the case of original and calcium soils but of these values only *CW* and *BEC* retain significance in the case of NaBrO treated soils. These differences must be ascribed to NaBrO treatment.

V. Discussion of results

Martin [1928] has pointed out that tropical soils have higher *CW* than non-tropical soils, pointing out that H and F. J. Warth obtained up to 28·1 per cent *CW* for Indian laterites while Lacrois found up to 30 per cent in French Guinea and Koert up to 27·9 per cent at Amani, Tanganyika. Martin also pointed out that *CW* is inversely proportional to the $\frac{SiO_2}{Al_2O_3}$ ratio in the clay if ferric oxide is constant. Coutts [1929] from his studies on Natal soils gave the following equation connecting *SP* and *L 1* :—

$$SP = 2{\cdot}05\ L\,1 + 17{\cdot}6 \pm 3{\cdot}1$$

and the relatively poor fit of this equation may possibly be due to the fact that the soils were of varying degrees of saturation. The fact that *CW* or *L 1* is reduced by replacement of H ions by Ca agrees with Jenny's contention [1932] that part of the combined water in H systems is the " potential water molecules " formed by exchangeable H ions and the OH ions in the alumino-silicate crystal lattice framework. The partial correlation coefficients detailed in Tables IV--VI leave no room for doubt that both *SP* and *R* are directly related to *CW* and that, other things being equal, not only will *SP* and *R* tend to be greater with tropical than with non-tropical soils but in general both *SP* and *R* will be higher in sour than in neutral or calcareous soils.

The multiple correlation coefficients for the equations given in Tables II and III are all very significant but the application of the equations to the calculation of *SP* and *R* shows that while *SP* may be calculated satisfactorily, the calculated values of *R* in many cases differ so widely from the actual values that the equations for *R* are of little or no practical use. This is clearly shown in Appendix Table VIII. The fact that *SP* may be fairly accurately calculated in soils containing only one replaceable base whereas *R* cannot be satisfactorily calculated, in spite

of the higher multiple correlation coefficients, is to be ascribed to the fact that *SP* includes about sixteen per cent interstitial water which is included within the *SP* values, so rendering errors between actual and calculated values of a lower order than is the case for *R* which includes no such interstitial water.

For practical use the calcium soils offer advantages over the NaBrO treated calcium soils. Reference to Appendix Table VII-A shows that equations A (2) (a) and A (2) (b) give almost identical results, the maximum difference in the calculated *SP* values for any soil being only 0·50. Both equations break down in one case only, *e.g.* for soil B. 624 which is a very sandy soil in which the determination of *SP* is difficult and almost meaningless. Since equations A (2) (a) and A (2) (b) give practically identical results there is no real advantage in expressing *L 1* separately as organic carbon and *CW*. In equation A (2) (b) the partial correlation between $\frac{SiO_2}{R_2O_3}$ and *SP* is of a low order only, *viz.* $r = 0{\cdot}2924$ with *P* about 0·1. Hence the equation may not be used for calculating $\frac{SiO_2}{R_2O_3}$. Also, if $\frac{SiO_2}{R_2O_3}$ be omitted, the equation further simplifies to

$$SP = 2{\cdot}637\ L\,1 + 23{\cdot}98$$

the correlation between *SP* and *L 1* having the highly significant value of 0·8672. Hence in soils saturated with calcium and containing no extreme ratios of $\frac{SiO_2}{R_2O_3}$, provided such soils are not extremely sandy, this equation fits very well. When applied to the corresponding soils in this case, twenty-seven soils have an error less than ten per cent, nine between ten and twenty per cent and two have an error greater than twenty per cent. The last two cases occur with soils B. 624 and B. 951. The sandy nature of B. 624 has already been referred to while B. 951 has more non-humified organic matter than any of the soils used although whether this is the cause of the difference between actual and calculated *SP* values is not known.

On the whole, therefore, the information yielded by determination of *SP* appears to offer no advantages over that given by *L 1* and since *L 1* is a much better controlled determination, less subject to personal errors, *L 1* is to be preferred to *SP*.

The case of *R* is by no means conclusive. So far as information yielded by the parameters used is concerned, *SP* and *R* seem to differ fundamentally although both are influenced largely by *CW* in the same direction. Thus organic carbon seems to be without effect on *R* but influences *SP* while carbon dioxide and *BEC* affect *R* but not *SP*. Of the parameters which affect *R*, *CE*, *CW* and $\frac{SiO_2}{R_2O_3}$ are in turn known to be correlated to *BEC* and hence it appears that *R* is much more influenced by *BEC* than *SP*. The fact that *BEC* alone is not adequate to determine *R* is possibly due to imperfections in the method used to determine *BEC*.

Also the substitution of $\frac{SiO_2}{Al_2O_3}$ (molr.) for $\frac{SiO_2}{R_2O_3}$ (molr.) might possibly simplify the relationships but there is at present no possibility of re-examining the work on these lines. Perhaps the most surprising part of the work is the fact that organic carbon is without influence on *R*. This emerges from treated and untreated soils, all three cases showing a negligible partial correlation between *R* and organic carbon. Although *R* appears to depend upon *BEC* and parameters related to *BEC* and also to the amount of carbonates present in the soil, the relationships obviously require further examination. Certain of the determinations, especially organic carbon and *CW* are approximate only and as a result the dependence of *R* upon *CE*, *CW*, carbon dioxide, etc. may be obscured. The small errors due to use of parameters based on air-dry soil and in other cases on oven-dry soil may also possibly contribute to the same result.

VI. Conclusions and summary

1. In an examination of the effect of clay, carbonates, organic carbon, combined water, $\frac{SiO_2}{R_2O_3}$ (molecular) in clay and base-exchange capacity on moisture at the Sticky Point and *R*, in untreated soils and in the same soils saturated with calcium, with and without removal of humified organic matter, considerable differences in behaviour of *SP* and *R* have been found.

2. Cold NaBrO solution removed about forty per cent organic matter on the average and was as successful with calcareous as with sour soils whereas hydrogen peroxide is not successful in removing organic matter from these soils.

3. The combined water was reduced on the average by about twenty per cent by saturating the soils with calcium ions. Excluding the group of calcareous soils, the reduction on the remainder was 34·7 per cent. The reduction is attributed to the fact that sour soils on ignition lose water by combination of exchangeable hydrogen and hydroxyl ions from the alumino-silicate crystal lattice framework. If this exchangeable H′ is replaced by calcium, such loss cannot occur.

4. Sticky Point moisture appears to vary with organic carbon and combined water (or loss on ignition) so long as calcium is the only replaceable base. Under these conditions r L 1. S P = 0·8672 and the Sticky Point moisture may be calculated with fair accuracy from the equation

$$SP = 2\ 637\ L\ 1 + 23{\cdot}98$$

The equation is not very accurate for very sandy soils and may fail if the soils have very high or very low $\frac{SiO_2}{R_2O_3}$ ratios.

5. Although *R* varies with combined water to a great extent as in the case of *SP*, it appears to depend largely upon base-exchange capacity or values known to be related to the base-exchange capacity. Carbonates and the $\frac{SiO_2}{R_2O_3}$ ratio may also affect *R* and further examination of this value is required.

Professor P. C. Mahalanobis has pointed out that application of the method of analysis of variance offers advantages in studying the relative importance of the various parameters at each stage of the correlational analysis. Owing to his transfer to other work the writer has had no opportunity to re-examine the work on these lines. Mr. E. G. Lewis, Lecturer in Mathematics, Rangoon University, has independently repeated the examination of the data for untreated and calcium soils utilizing the method of analysis of variance. His results check those quoted in the paper so closely that the regression equations are practically identical. To these two gentlemen, as well as to several assistants in the chemical section of the Agricultural College, Mandalay, the writer desires to express his thanks and particularly to Mr. M. Menon, B.A., who has devoted a great deal of his own time to checking of arithmetical work.

It would have been of advantage had the standard error of the regression coefficients been calculated. Unfortunately, for reasons previously stated, the writer is unable to devote any more time to the subject and the paper has necessarily to be published in its present form.

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Appendix

TABLE I

Original soils

Soil No.	Clay equivalent	CO_2 as carbonate	Organic carbon	Combined water	$\frac{SiO_2}{R_2O_3}$ (Molr.)	$\frac{SiO_2}{Al_2O_3}$ (Molr.)	*BEC*	*S P* water	*R*
Hmawbi good 0-6″ . .	31·01	0·03	0·980	2·528	2·48	2·71	10·50	41·23	2·099
B 610	56·17	0·04	1·558	9·797	2·16	2·62	21·00	43·95	4·435
B 616 . . .	67·59	0·04	2·198	11·454	2·22	2·56	27·83	49·03	5·537
B 618	49·81	0·06	1·784	8·176	2·40	2·90	20·17	43·66	3·869
B 624	22·80	0·04	0·904	2·946	2·61	2·93	5·42	22·85	1·328
B 951	75·85	0·07	2·763	7·706	1·57	1·99	19·75	49·47	3·937

TABLE I—*contd.*

Soil No.	Clay equivalent	CO_2 as carbonate	Organic carbon	Combined water	$\frac{SiO_2}{R_2O_3}$ (Molr.)	$\frac{SiO_2}{Al_2O_3}$ (Molr.)	*BEC*	*S P* water	*R*
B 957	73·66	0·10	2·915	4·680	1·85	2·26	19·83	53·28	3·905
B988	37·99	0·03	1·306	2·545	2·05	2·51	7·92	34·38	1·576
B 992	50·85	0·02	1·753	2·963	2·55	3·19	14·66	44·32	2·528
B 999	36·86	0·02	1·501	2·241	2·60	3·21	11·83	34·75	1·581
B 1010	44·83	0·02	1·177	2·750	2·68	3·26	10·33	40·44	1·772
B 1015	44·00	0·04	1·637	3·664	2·42	2·89	8·67	41·81	1·903
B 1031	36·26	0·01	1·237	2·051	2·60	3·13	10·25	30·74	1·589
B 1129	22·50	0·04	1·646	8·847	1·55	1·77	9·17	48·53	2·506
B 1131	30·12	0·04	2·239	10·279	1·55	1·75	10·33	56·16	3·105
B 1133	32·39	0·07	1·015	11·545	1·90	2·25	13·00	55·48	3·177
B 1135	27·13	0·06	1·287	12·690	1·55	1·75	8·83	49·23	3·164
B 1137	20·30	0·04	1·080	4·426	1·42	1·62	7·42	34·12	1·351
B 1139	12·65	0·11	0·777	3·707	1·92	2·39	7·25	31·53	1·359
B 1141	9·70	0·02	0·556	2·719	1·80	2·25	5·42	27·95	0·993
B 1143	15·51	1·12	0·879	4·412	1·94	2·36	6·17	46·14	1·535
Nanda Tank . . .	50·71	9·082	2·146	6·188	2·37	2·87	21·09	46·32	5·266
Mandalay 1 . . .	53·34	3·488	0·830	4·501	2·70	3·35	16·00	36·42	5·857
„ 10 . . .	65·71	2·887	0·633	5·549	2·49	3·02	27·50	40·84	6·418
„ 13 . . .	62·18	6·378	2·275	5·004	3·53	4·30	10·50	54·31	5·916
„ 21 . . .	34·34	1·101	1·137	5·183	2·11	2·58	20·08	41·78	3·983
„ 27 . . .	46·07	6·733	1·295	3·965	2·19	2·59	15·67	37·52	3·339
„ 28 . . .	56·01	1·207	0·890	2·672	2·38	2·88	27·67	38·00	5·473
„ 33 . . .	41·50	2·399	0·935	3·970	2·35	2·90	16·17	34·34	3·436
„ 34 . . .	39·51	1·134	0·684	4·706	2·37	2·90	19·00	30·94	3·597
„ 35 . . .	51·62	1·025	1·007	4·029	2·14	2·59	22·08	34·62	4·528
„ 36 . . .	54·04	0·871	0·933	3·984	2·03	2·48	23·58	36·38	4·623
„ 38 . . .	41·57	4·094	0·341	4·480	2·01	2·43	14·67	32·15	3·353
Old 28 . . .	52·28	6·360	2·644	5·763	1·99	2·36	22·50	38·33	4·656
Old 57 . . .	68·69	4·160	4·017	4·346	3·43	4·28	11·17	63·80	6·270
Singaing 1 . . .	34·49	3·012	0·894	2·285	2·17	2·74	11·50	37·87	1·826
„ 2 . . .	33·95	2·240	0·556	2·169	2·41	2·97	13·33	32·39	2·187
„ 3 . . .	52·89	2·764	0·721	1·909	2·56	3·15	19·33	37·18	3·724
Means	43·08	1·604	1·398	5·074	2·24	2·70	14·94	40·85	3·361

TABLE II

$CaCl_2$ treated soils

Soil No.	CO_2 as carbonate	Organic carbon	Combined water	Sticky point water	*R*
Hmawbi Good	0·09	0·920	2·032	38·05	2·292
610	0·03	1·520	3·549	46·72	4·434
616	0·06	2·182	4·130	51·67	5·542
618	0·07	1·724	4·941	44·31	3·815
624	0·01	0·899	1·749	21·15	1·141
951	0·13	3·364	8·574	51·49	4·722
957	0·11	2·900	6·427	53·85	4·175
988	0·05	1·231	2·482	33·41	2·025
992	0·17	1·625	3·336	42·26	2·921
999	0·04	1·473	2·420	38·30	2·062
1010	0·08	1·073	2·466	38·56	1·782
1015	0·05	1·624	2·946	40·19	1·908
1031	0·04	1·197	2·867	33·72	1·535
1129	0·04	1·660	5·125	47·37	2·191
1131	0·11	2·162	6·099	54·87	2·750
1133	0·04	1·834	5·124	50·88	2·861
1135	0·06	2·321	6·750	47·03	2·783
1137	0·11	1·021	2·208	34·05	1·337
1139	0·08	0·833	2·457	31·85	1·203
1141	0·07	0·539	1·689	27·93	0·891
1143	1·10	0·936	2·368	34·27	1·339
N. T.	9·11	3·135	4·450	47·16	4·999
Mandalay 1	3·94	1·190	4·628	41·36	5·265
„ 10	2·99	0·912	5·815	45·00	6·508
„ 13	7·14	1·763	7·375	55·32	5·663
„ 21	1·20	1·808	4·323	41·85	3·830
„ 27	7·03	1·555	3·251	37·64	3·195
„ 28	1·28	1·208	3·605	44·80	5·407
„ 33	2·38	1·069	3·936	38·41	3·399
„ 34	1·28	0·696	2·305	32·22	3·635
„ 35	1·54	1·249	3·222	40·32	4·457
„ 36	1·15	0·958	5·017	40·81	4·532
„ 38	4·46	0·599	3·725	34·65	3·257
Old 28	6·33	2·304	5·445	45·84	4·988
Old 57	5·81	3·158	6·618	65·94	6·825
Singaing 1	3·19	0·841	3·324	38·56	2·421
„ 2	2·47	0·523	2·283	32·78	2·558
„ 3	3·29	0·593	3·433	40·16	4·279
Means	1·77	1·489	4·013	41·70	3·393

TABLE III

NaBrO treated calcium soils

Soil No.	CO_2 as carbonate	Organic carbon	Combined water	Sticky point moisture	*R*
Hmawbi good 0-6″	0·18	0·486	2·342	37·90	2·026
B 610	0·34	0·729	4·510	46·48	4·226
B 616	0·30	1·175	5·161	50·91	5·279
B 618	0·21	0·923	3·846	42·00	3·746
B 624	0·10	0·427	1·200	23·87	1·047
B 951	0·42	2·533	5·865	48·58	4·100
B 957	0·41	2·208	5·264	53·05	3·849
B 988	0·20	0·717	2·399	31·23	1·659
B 992	0·22	1·079	2·643	40·10	2·576
B 999	0·19	0·922	1·508	32·75	1·749
B 1010	0·15	0·674	2·488	38·04	1·905
B 1015	0·15	1·125	3·174	39·53	2·120
B 1031	0·59	0·338	3·953	29·98	1·590
B 1129	0·20	1·223	5·064	45·66	2·503
B 1131	0·19	1·783	6·063	51·72	3·195
B 1133	0·19	1·471	4·805	52·48	3·221
B 1135	0·27	1·883	5·723	46·52	3·219
B 1137	0·11	0·707	1·852	32·42	1·370
B 1139	0·22	0·508	1·965	29·83	1·165
B 1141	0·13	0·300	1·451	23·65	0·753
B 1143	1·22	0·630	2·062	32·19	1·208
Nanda Tank	9·75	1·148	5·910	43·77	4·293
Mandalay 1	4·38	0·511	3·976	41·05	5·152
„ 10	3·18	0·399	4·681	46·47	5·995
„ 13	7·34	0·694	6·178	55·61	5·262
„ 21	1·44	0·812	3·728	38·96	3·149
„ 27	7·01	0·756	3·240	36·19	2·793
„ 28	1·52	0·506	3·582	42·20	4·481
„ 33	2·87	0·396	4·179	34·48	2·665
„ 34	1·43	0·333	2·719	31·34	2·842
„ 35	1·30	0·513	3·364	39·19	3·568
„ 36	1·17	0·486	3·930	37·18	3·794
„ 38	4·23	0·380	3·706	33·22	3·195
Old 28	6·76	0·679	4·675	44·50	4·031
Old 57	6·22	1·093	5·691	63·86	5·719
Singaing 1	3·42	0·299	3·034	34·45	2·029
„ 2	2·75	0·230	2·760	31·83	2·449
„ 3	3·11	0·376	3·304	40·44	4·010
Means	1·944	0·828	3·737	40·10	3·104

TABLE IV

Original soils (untreated)

(*A*) *SP* Correlations

Put *SP* = 1
CE = 2
CO_2 = 3
Organic carbon = 4
CW = 5
BEC = 6
$\frac{SiO_2}{R_2O_3}$ = 7

(*B*) *R* Correlations

Put *R* = 1
CE = 2
CO_2 = 3
Organic carbon = 4
CW = 5
BEC = 6
$\frac{SO}{R_2O_3}$ = 7

A

$r_{12} = 0{\cdot}4468$ $r_{23} = 0{\cdot}2592$ $r_{34} = 0{\cdot}1835$ $r_{45} = 0{\cdot}3471$ $r_{56} = 0{\cdot}2004$
$r_{67} = 0{\cdot}03922$
$r_{13} = 0{\cdot}09683$ $r_{24} = 0{\cdot}5720$ $r_{35} = -0{\cdot}1405$ $r_{46} = 0{\cdot}1109$ $r_{57} = -0{\cdot}4072$
$r_{14} = 0{\cdot}7447$ $r_{25} = 0{\cdot}1629$ $r_{36} = 0{\cdot}2297$ $r_{47} = 0{\cdot}1603$
$r_{15} = 0{\cdot}6112$ $r_{26} = 0{\cdot}7266$ $r_{37} = 0{\cdot}3384$
$r_{16} = 0{\cdot}1136$ $r_{27} = 0{\cdot}3496$
$r_{17} = -0{\cdot}1629$
$r_{12{\cdot}34567} = 0{\cdot}4041$
$r_{13{\cdot}24567} = 0{\cdot}2374$
$r_{14{\cdot}23567} = 0{\cdot}4126$
$r_{15{\cdot}23467} = 0{\cdot}5204$
$r_{16{\cdot}23457} = -0{\cdot}3859$
$r_{17{\cdot}23456} = -0{\cdot}3950$

B

$r_{12} = 0{\cdot}7947$ $r_{23} = 0{\cdot}2892$ $r_{34} = 0{\cdot}1835$ $r_{45} = 0{\cdot}3471$ $r_{56} = 0{\cdot}2004$
$r_{67} = 0{\cdot}03922$
$r_{13} = 0{\cdot}4599$ $r_{24} = 0{\cdot}5720$ $r_{35} = -0{\cdot}1405$ $r_{46} = 0{\cdot}1109$ $r_{57} = -0{\cdot}4072$
$r_{14} = 0{\cdot}4299$ $r_{25} = 0{\cdot}1629$ $r_{36} = 0{\cdot}2297$ $r_{47} = 0{\cdot}1603$
$r_{15} = 0{\cdot}3570$ $r_{26} = 0{\cdot}7266$ $r_{37} = 0{\cdot}3384$
$r_{16} = 0{\cdot}7546$ $r_{27} = 0{\cdot}3496$
$r_{17} = 0{\cdot}3172$
$r_{12{\cdot}34567} = 0{\cdot}2652$

$r_{13 \cdot 24567} = 0 \cdot 5208$
$r_{14 \cdot 23567} = 0 \cdot 03149$
$r_{15 \cdot 23467} = 0 \cdot 6214$
$r_{16 \cdot 23457} = 0 \cdot 4974$
$r_{17 \cdot 23456} = 0 \cdot 4839$

TABLE V

$CaCl_2$ treated soils

(*A*) *SP* Correlations
Put *SP* = 1
CE = 2
CO_2 = 3
Organic carbon = 4
CW = 5
BEC = 6
$\frac{SiO_2}{R_2O_3}$ = 7

(*B*) *R* Correlations
Put *R* = 1
CE = 2
CO_2 = 3
Organic carbon = 4
CW = 5
BEC = 6
$\frac{SiO_2}{R_2O_3}$ = 7

A

$r_{12} = 0 \cdot 6555$ $r_{23} = 0 \cdot 3200$ $r_{34} = 0 \cdot 2260$ $r_{45} = 0 \cdot 7210$ $r_{56} = 0 \cdot 3367$
$r_{67} = 0 \cdot 03922$
$r_{13} = 0 \cdot 2453$ $r_{24} = 0 \cdot 5250$ $r_{35} = 0 \cdot 2596$ $r_{46} = 0 \cdot 2211$ $r_{57} = -0 \cdot 0370$
$r_{14} = 0 \cdot 7695$ $r_{25} = 0 \cdot 6156$ $r_{36} = 0 \cdot 2174$ $r_{47} = -0 \cdot 07098$
$r_{15} = 0 \cdot 8139$ $r_{26} = 0 \cdot 7266$ $r_{37} = 0 \cdot 3974$
$r_{16} = 0 \cdot 3697$ $r_{27} = 0 \cdot 3496$
$r_{17} = 0 \cdot 1264$
$r_{12 \cdot 34567} = -0 \cdot 01485$
$r_{13 \cdot 24567} = -0 \cdot 1559$
$r_{14 \cdot 23567} = 0 \cdot 4910$
$r_{15 \cdot 23467} = 0 \cdot 5483$
$r_{16 \cdot 23457} = 0 \cdot 1511$
$r_{17 \cdot 23456} = 0 \cdot 2924$

B

$^{r}12 = 0{\cdot}8652$ $^{r}23 = 0{\cdot}3200$ $^{r}34 = 0{\cdot}2260$ $^{r}45 = 0{\cdot}7210$ $^{r}56 = 0{\cdot}3367$
$^{r}67 = 0{\cdot}03922$

$^{r}13 = 0{\cdot}4923$ $^{r}24 = 0{\cdot}5250$ $^{r}35 = 0{\cdot}2596$ $^{r}46 = 0{\cdot}2211$ $^{r}57 = -0{\cdot}0370$

$^{r}14 = 0{\cdot}4520$ $^{r}25 = 0{\cdot}6156$ $^{r}36 = 0{\cdot}2174$ $^{r}47 = -0{\cdot}07098$

$^{r}15 = 0{\cdot}6549$ $^{r}26 = 0{\cdot}7266$ $^{r}37 = 0{\cdot}3974$

$^{r}16 = 0{\cdot}7739$ $^{r}27 = 0{\cdot}3496$

$^{r}17 = 0{\cdot}3676$

$^{r}12{\cdot}34567 = 0{\cdot}1760$

$^{r}13{\cdot}24567 = 0{\cdot}3843$

$^{r}14{\cdot}23567 = -0{\cdot}06015$

$^{r}15{\cdot}23467 = 0{\cdot}6442$

$^{r}16{\cdot}23457 = 0{\cdot}7137$

$^{r}17{\cdot}23456 = 0{\cdot}5319$

TABLE VI

NaBrO treated calcium soils

(*A*) *SP* Correlations	(*B*) *R* Correlations
Put *SP* = 1	Put *R* = 1
CE = 2	*CE* = 2
CO_2 = 3	CO_2 = 3
Organic carbon = 4	Organic carbon = 4
CW = 5	*CW* = 5
BEC = 6	*BEC* = 6
$\frac{SiO_2}{R_2O_3} = 7$	$\frac{SiO_2}{R_2O_3} = 7$

A

$^{r}12 = 0{\cdot}6790$ $^{r}23 = 0{\cdot}2984$ $^{r}34 = -0{\cdot}2213$ $^{r}45 = 0{\cdot}5822$ $^{r}56 = 0{\cdot}4030$
$^{r}67 = 0{\cdot}03922$

$^{r}13 = 0{\cdot}2342$ $^{r}24 = 0{\cdot}3192$ $^{r}35 = 0{\cdot}3343$ $^{r}46 = 0{\cdot}02301$ $^{r}57 = -0{\cdot}009638$

$^{r}14 = 0{\cdot}6005$ $^{r}25 = 0{\cdot}6028$ $^{r}36 = 0{\cdot}2288$ $^{r}47 = -0{\cdot}3987$

$^{r}15 = 0{\cdot}8679$ $^{r}26 = 0{\cdot}7266$ $^{r}37 = 0{\cdot}4111$

$^{r}16 = 0{\cdot}3863$ $^{r}27 = 0{\cdot}3496$

$r_{17} = 0{\cdot}1402$

$r_{12{\cdot}34567} = 0{\cdot}07427$

$r_{13{\cdot}24567} = -0{\cdot}1248$

$r_{14{\cdot}23567} = 0{\cdot}3145$

$r_{15{\cdot}23467} = 0{\cdot}6904$

$r_{16{\cdot}23457} = 0{\cdot}07186$

$r_{17{\cdot}23456} = 0{\cdot}3320$

B

$r_{12} = 0{\cdot}8448$ $r_{23} = 0{\cdot}2984$ $r_{34} = -0{\cdot}2213$ $r_{45} = 0{\cdot}5822$ $r_{56} = 0{\cdot}4030$
$r_{67} = 0{\cdot}03922$

$r_{13} = 0{\cdot}4547$ $r_{24} = 0{\cdot}3192$ $r_{35} = 0{\cdot}3343$ $r_{46} = 0{\cdot}02301$ $r_{57} = -0{\cdot}009638$

$r_{14} = 0{\cdot}2161$ $r_{25} = 0{\cdot}6028$ $r_{36} = 0{\cdot}2288$ $r_{47} = -0{\cdot}3987$

$r_{15} = 0{\cdot}7517$ $r_{26} = 0{\cdot}7266$ $r_{37} = 0{\cdot}4111$

$r_{16} = 0{\cdot}7290$ $r_{27} = 0{\cdot}3496$

$r_{17} = 0{\cdot}3101$

$r_{12{\cdot}34567} = 0{\cdot}3445$

$r_{13{\cdot}24567} = 0{\cdot}1015$

$r_{14{\cdot}23567} = -0{\cdot}1845$

$r_{15{\cdot}23467} = 0{\cdot}6613$

$r_{16{\cdot}23457} = 0{\cdot}3642$

$r_{17{\cdot}23456} = 0{\cdot}1949$

TABLE VII-A

Sticky Point moisture—actual and calculated values

Soil No.	Original soils			$CaCl_2$ treated soils					NaBrO treated Ca soils				
	Actual S. P.	Calculated S. P. (Equation A1)	Difference Col. 2 — Col. 3	Actual S. P.	Calculated S. P. [Equation A2(*a*)]	Difference Col. 5— Col. 6	Calculated S. P. [Equation, A2 (*b*)]	Difference Col. 5— Col. 8	Actual S. P.	Calculated S. P. [Equation A3(*a*)]	Difference Col. 10 — Col. 11	Calculated S. P. [Equation A3(*b*)]	Difference Col. 10— Col. 13
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
Hmawbi 0-6" .	41·23	35·11	+6·12	38·05	34·45	+3·60	34·52	+3·53	37·90	33·90	+4·00	34·12	+3·78
B 610 . .	43·95	48·76	—4·81	46·72	40·29	+6·43	40·36	+6·36	46·48	42·20	+4·28	41·79	+4·69
B 616 . .	49·03	54·02	—4·99	51·67	45·04	+6·43	45·26	+6·41	50·91	47·73	+3·18	47·59	+3·32
B 618 . .	43·66	47·28	—3·62	44·31	45·82	—1·51	45·79	—1·48	42·00	42·02	—0·02	42·14	—0·14
B 624 . .	22·85	36·19	—13·34	21·15	33·99	—12·84	34·09	—12·94	23·87	29·71	—5·84	30·30	—6·43
B 951 . .	49·47	52·99	—3·52	51·49	60·52	—9·03	60·55	—9·06	48·58	54·56	—5·98	55·28	—6·70
B 957 . .	53·28	49·25	+4·03	53·85	53·41	+0·44	53·57	+0·28	53·05	51·90	+1·15	52·62	+0·43
B 988 . .	34·38	37·50	—3·12	33·41	35·70	—2·29	35·81	—2·40	31·23	32·96	—1·73	33·20	—1·97
B 992 . .	44·32	41·03	+3·29	42·26	41·42	+0·84	41·56	+0·70	40·10	38·92	+1·18	39·68	+0·42
B 999 . .	34·75	37·83	—3·08	38·30	38·37	—0·07	38·58	—0·28	32·75	33·72	—0·97	34·72	—1·97
B 1010 . .	40·44	38·73	+1·71	38·56	36·97	+1·59	37·04	+1·52	38·04	36·73	+1·31	37·17	+0·87
B 1015 . .	41·81	42·28	—0·47	40·19	39·92	+0·27	40·11	+0·08	39·53	40·57	—1·04	41·14	—1·61
B 1031 . .	30·74	36·65	—5·91	33·72	38·38	—4·66	38·43	—4·71	29·98	40·26	—10·28	39·85	—9·87
B 1129 . .	48·53	45·68	+2·85	47·37	43·32	+4·05	43·23	+4·14	45·66	43·72	+1·94	43·36	+2·30
B 1131 . .	56·16	51·50	+4·66	54·87	48·25	+6·62	48·20	+6·67	51·72	50·96	+0·76	50·84	+0·88
B 1133 . .	55·48	47·72	+7·76	50·88	45·22	+5·66	45·19	+5·69	52·48	46·12	+6·36	42·26	+6·22
B 1135 . .	49·23	50·73	—1·50	47·03	50·76	—3·73	50·68	—3·65	46·52	50·16	—3·64	50·25	—3·73
B 1137 . .	34·12	35·83	—1·71	34·05	31·99	+2·06	32·05	+2·00	32·42	27·04	+5·38	27·15	+5·27
B 1139 . .	31·53	33·38	—1·85	31·85	33·44	—1·59	33·42	—1·57	29·83	29·26	+0·57	29·37	+0·46

TABLE VII-A—*contd.*

Soil No.	Original soils			$CaCl_2$ treated soils					NaBrO treated Ca soils				
	Actual S. P.	Calculated S. P. (Equation A1)	Difference Col. 2—Col. 3	Actual S. P.	Calculated S. P. [Equation A2(*a*)]	Difference Col. 5—Col. 6	Calculated S. P. [Equation, A2(*b*)]	Difference Col. 5—Col. 8	Actual S. P.	Calculated S. P. [Equation A3(*a*)]	Difference Col. 10—Col. 11	Calculated S. P [Equation A3(*b*)]	Difference Col. 10—Col. 13
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
B 1141 . .	27·95	30·64	—2·69	27·93	29·62	—1·69	29·60	—1·67	23·65	25·30	—1·65	25·30	—1·65
B 1143 . .	46·14	35·76	+10·38	34·27	33·72	+0·55	33·74	+0·53	32·19	30·46	+1·73	30·67	+1·52
Nanda Tank. .	46·32	45·66	+0·66	47·16	50·66	—3·50	51·16	—4·00	43·77	51·47	—7·70	51·13	—7·36
Mandalay 1 . .	36·42	39·17	—2·75	41·36	43·54	—2·18	43·37	—2·01	41·05	41·92	—0·87	41·73	—0·68
„ 10 .	40·84	37·38	+3·46	45·00	44·91	+0·09	44·49	+0·51	46·47	42·91	+3·56	42·27	+4·20
„ 13 .	54·31	51·83	+2·48	55·32	56·35	—1·03	56·03	—0·71	55·61	56·68	—1·07	56·33	—0·72
„ 21 .	41·78	36·70	+5·08	41·85	43·56	—1·71	43·62	—1·77	38·96	39·22	—0·26	39·14	—0·18
„ 27 .	37·52	38·62	—1·10	37·64	39·72	—2·08	39·84	—2·20	36·19	37·39	—1·20	37·46	—1·27
„ 28 .	38·00	32·51	+5·49	44·80	39·76	+5·04	39·72	+5·08	42·20	38·45	+3·75	38·23	+3·97
„ 33 .	34·34	36·47	—2·13	38·41	39·96	—1·55	39·84	—1·43	34·48	40·06	—5·58	39·51	—5·03
„ 34 .	30·94	35·30	—4·36	32·22	33·86	—1·64	33·81	—1·59	31·34	33·91	—2·57	33·80	—2·46
„ 35 .	34·62	35·90	—1·28	40·32	38·12	+2·20	38·14	+2·18	39·19	36·22	+2·97	35·96	+3·23
„ 36 .	36·38	35·06	+1·32	40·81	41·43	—0·62	41·12	—0·31	37·18	37·71	—0·53	37·19	—0·01
„ 38 .	32·15	34·07	—1·92	34·65	36·20	—1·55	35·93	—1·28	33·22	36·08	—2·86	35·52	—2·30
Old 28 .	38·33	46·34	—8·01	45·84	48·49	—2·65	48·57	—2·73	44·50	41·59	+2·91	41·01	+3·49
„ 57 .	63·80	59·77	+4·03	65·94	60·15	+5·79	60·40	+5·54	63·86	56·42	+7·44	56·59	+7·27
Singaing 1 .	37·87	33·74	+4·13	38·56	36·68	+1·88	36·55	+2·01	34·45	33·83	+0·62	33·49	+0·96
„ 2 .	32·39	31·79	+0·60	32·78	33·15	—0·37	33·05	—0·27	31·83	33·72	—1·89	33·51	—1·68
„ 3 .	37·18	33·08	+4·10	40·16	37·13	+3·03	36·90	+3·26	40·44	37·63	+2·81	37·45	+2·99

TABLE VII-B

Values of R—*actual and calculated*

Soil No.	Original soils			$CaCl_2$ treated soils			Na BrO treated calcium soils				
	Actual R	Calculated R (Equation B1)	Difference Col. 2—Col. 3	Actual R	Calculated R (Equation B2)	Difference Col. 5—Col. 6	Actual R	Calculated R (Equation B3a)	Difference Col. 8 — Col. 9	Calculated R (Equation B3b)	Difference Col. 8—Col. 11
1	2	3	4	5	6	7	8	9	10	11	12
Hmawbi 0-6 in. . . .	2·099	2·212	—0·113	2·292	2·064	+0·228	2·026	1·895	+0·131	2·085	—0·059
B 610	4·435	4·854	—0·419	4·434	3·817	+0·617	4·226	4·191	+0·035	4·094	+0·132
B 616	5·537	6·269	—0·732	5·542	5·078	+0·464	5·279	5·224	+0·055	5·182	+0·097
B 618	3·869	4·709	—0·840	3·815	4·476	—0·661	3·746	3·675	+0·071	3·812	—0·066
B 624	1·328	1·746	—0·418	1·141	1·352	—0·211	1·047	0·871	+0·176	1·092	—0·045
B 951	3·937	3·450	+0·487	4·722	4·981	—0·259	4·100	5·276	—1·176	4·408	—0·308
B 957	3·905	3·191	+0·714	4·175	4·449	—0·274	3·849	4·970	—1·121	4·315	—0·466
B 988	1·576	1·275	+0·301	2·025	1·433	+0·592	1·659	1·980	—0·321	1·630	+0·029
B 992	2·528	2·994	—0·466	2·921	3·236	—0·315	2·576	2·888	—0·312	2·970	—0·394
B 999	1·581	2·499	—0·918	2·062	2·517	—0·455	1·749	1·823	—0·074	1·950	—0·201
B 1010	1·772	2·498	—0·726	1·782	2·405	—0·623	1·905	2·375	—0·470	2·452	—0·547
B 1015	1·903	2·114	—0·211	1·908	2·088	—0·180	2·120	2·525	—0·405	2·433	—0·313
B 1031	1·589	2·229	—0·640	1·535	2·463	—0·928	1·590	2·694	—1·104	3·008	—1·418
B 1129	2·506	2·142	+0·364	2·191	2·121	+0·070	2·503	2·636	—0·143	2·486	+0·017
B 1131	3·105	2·615	+0·490	2·750	2·667	+0·083	3·195	3·357	—0·162	3·162	+0·033
B 1133	3·177	3·739	—0·562	2·861	3·018	—0·157	3·221	3·083	+0·138	3·075	+0·146
B 1135	3·164	2·919	+0·245	2·783	2·696	+0·087	3·219	3·036	+0·183	2·832	+0·387
B 1137	1·351	0·773	+0·578	1·337	0·631	+0·706	1·370	1·177	+0·193	0·627	+0·743
B 1139	1·359	1·370	—0·011	1·203	1·200	+0·003	1·165	0·973	+0·192	0·975	+0·190
B 1141	0·993	0·620	+0·373	0·891	0·524	+0·367	0·753	0·563	+0·190	0·436	+0·317

TABLE VII-B—*contd.*

Soil No.	Original soils			$CaCl_2$ treated soils			NaBrO treated calcium soils				
	Actual R	Calculated R (Equation B1)	Difference Col. 2—Col. 3	Actual R	Calculated R (Equation B2)	Difference Col. 5—Col. 6	Actual R	Calculated R (Equation B3a)	Difference Col. 8—Col. 9	Calculated R (Equation B3)	Difference Col. 8—Col. 11
1	2	3	4	5	6	7	8	9	10	11	12
B 1143	1·535	1·476	+0·059	1·339	1·139	+0·200	1·208	1·036	+0·172	0·976	+0·232
Nanda Tank	5·266	6·018	—0·752	4·999	5·341	—0·342	4·293	4·591	—0·298	4·894	—0·601
Mandalay 1	5·857	4·345	+1·512	5·265	4·469	+0·796	5·152	3·584	+1·568	3·788	+1·364
„ 10	6·418	5·834	+0·584	6·508	6·255	+0·253	5·995	4·952	+1·043	5·108	+0·887
„ 13	5·916	5·288	+0·628	5·663	5·904	—0·241	5·262	4·414	+0·848	5·132	+0·130
„ 21	3·983	3·861	+0·122	3·830	4·055	—0·225	3·149	3·139	+0·010	3·348	—0·199
„ 27	3·339	4·097	—0·758	3·195	3·709	—0·514	2·793	3·041	—0·248	2·917	—0·124
„ 28	5·473	4·792	+0·681	5·407	5·143	+0·264	4·481	4·217	+0·264	4·388	+0·093
„ 33	3·436	3·594	—0·158	3·399	3·713	+0·286	2·665	3·307	—0·642	3·497	—0·832
„ 34	3·597	3·954	—0·357	3·635	3·397	+0·238	2·842	2·828	+0·014	3·018	—0·176
„ 35	4·528	3·928	+0·600	4·457	3·985	+0·472	3·568	3·653	—0·085	3·558	+0·010
„ 36	4·623	3·960	+0·663	4·532	4·735	—0·203	3·794	4·048	—0·254	3·908	—0·114
„ 38	3·353	3·345	+0·008	3·257	3·296	—0·039	3·195	3·028	+0·167	2·868	+0·327
Old 28	4·656	5·131	—0·475	4·988	5·250	—0·262	4·031	4·227	—0·196	4·129	—0·098
„ 57	6·270	4·607	+1·663	6·825	5·469	+1·356	5·719	4·462	+1·257	4·953	+0·766
Singaing 1	1·826	2·435	—0·609	2·421	2·717	—0·296	2·029	2·344	—0·315	2·309	—0·280
„ 2	2·187	2·851	—0·674	2·558	2·744	—0·186	2·449	2·327	+0·122	2·515	—0·066
„ 3	3·724	3·952	—0·228	4·279	4·278	+0·001	4·010	3·502	+0·512	3·632	+0·378

TABLE VIII

Differences between actual and calculated values for S. P. *and* R

Error in *S. P.*	Untreated soils	Calcium soils		NaBrO treated calcium soils	
	[Equation A1]	(*a*) [Equation A (2) (*a*)]	(*b*) [Equation A(2) (*b*)]	(*a*) [Equation A(3) (*a*)]	(*b*) [Equation A(3) (*b*)]
Less than 10 per cent	25	31	30	29	29
10-20 per cent	11	6	7	7	7
20 per cent	2	1	1	2	2
Error in *R*	Untreated soils	Calcium soils		NaBrO treated calcium soils	
	[Equation B (1)]	[Equation B (2)]		(*a*) [Equation B(3) (*a*)]	(*b*) [Equation B (3) (*b*)]
Less than 10 per cent	11	21		19	20
10-20 per cent	15	11		10	12
20 per cent	12	6		9	6

RAPID DETERMINATION OF SOIL-MOISTURE BY DRYING THE SOIL OVER BUNSEN BURNER*

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In soil investigations it is often necessary to have a quick estimate of moisture with a fair amount of accuracy in soil samples brought fresh from the field. Methods† suggested by Bouyoucos [1926] and Nitzch [1927] for the rapid determination of soil moisture are one and the same in essence and consist in adding a definite volume of alcohol of known specific gravity to a definite weight of moist soil, shaking the mixture for a few minutes and finally determining the specific gravity of the semi-clear liquid obtained on standing or by filtration. These methods are based on the assumption that all the moisture in the soil goes to dilute the added alcohol the specific gravity of which is not affected in any other way.

The author tried this alcohol method on a number of heavy Rothamsted soils in connection with another investigation reported elsewhere [1932] in which a rapid determination of moisture content in a number of fresh soil samples had to be made almost daily for eighteen months. It was found that in the majority of cases the percentage of moisture obtained by the alcohol method was lower than the oven-dried percentage by more than 1·5 per cent and in some cases by five to seven per cent if the soil happened to be very sticky‡. The shaking of mixture of alcohol and soil in an end-over-end-shaker from thirty minutes to one hour gave better results, but the values were still more than 1-1·5 per cent too low. Shaking for longer periods was not tried for obvious reasons.**

* This work was carried out at the Rothamsted Experimental Station while the author was in England during 1927-29. Owing to pressure of work he could not write out and communicate the paper for publication so long.

† Recently Obaton [1935] has elaborated a rapid method for determining soil moisture from the measurement of electrical resistance in soil using alternating current. Complications arising out of the presence of varying amount and nature of colloidal materials in soil are, however, likely to make this method unsuitable for general use. Obaton himself observed that colloid soil behaved differently from sandy soil.

‡ Later on Smith and Flint [1930] also tried the alcohol method and found it to be unreliable for fine textured soils and those containing alkali.

** Bouyoucos [1931] subsequently admitted the defect in his original method and tried to improve it by introducing mechanical dispersion of soil by a motor-driven stirrer.

If therefore became necessary to try other methods and the possibility of drying the soil directly over Bunsen burner was tested. A quantity of moist soil (between ten and fifteen grams. was found to be very convenient) was taken in a fused silica basin and placed over a rose burner (preferably a mushroom head as sold by Messrs. A Gallenkamp & Co. Ltd., specification No. 214) at a height of about two to three inches from the flames so as to have the heat evenly distributed at the bottom of the basin. The soil was constantly stirred and small clods broken, as drying proceeded, with thin metal rod having a glass or wooden handle. The colour of the soil changed due to loss of moisture and it was easy to detect when the soil was dry without burning it. After the soil was apparently dry it was still kept over the flame for half a minute or so to ensure complete loss of moisture. It takes three to six minutes for a soil to dry completely. It is advisable to remove the basin off the flame for about twenty seconds once or twice during the heating to avoid burning of the soil. It was found that percentages of moisture of the Rothamsted soils obtained in this way were seldom higher than those obtained by drying overnight in a steam oven at 98°-99° C. by more than one per cent. With a little practice the difference averaged round about 0·5 per cent by the two methods, the percentages by the Bunsen burner method being generally higher. It has been possible to determine moisture content of ten different soils weighing, drying, calculation etc., all complete in one hour and fifteen minutes. Table I below gives the percentages of several Rothamsted soils calculated on the dry basis and determined simultaneously by the three methods *viz.*, by drying over Bunsen burner, by drying in a steam oven for twenty-four hours, and by alcohol. All percentages of moisture by the alcohol method were determined after shaking the mixture of soil and alcohol vigorously for one minute and then filtering a portion of the supernatant liquid after half an hour's standing except those which are followed by the figure (½h.) or (1h.) in which case the mixture was shaken for half or one hour respectively.

TABLE I

Showing the comparison between percentages of moisture of several heavy Rothamsted soils as determined by three different methods

Soil No.	Percentage of moisture as determined by			Soil No.	Percentage of moisture as determined by		
	Drying over Bunsen burner	Drying in steam oven	Alcohol method		Drying over Bunsen burner	Drying in steam oven	Alcohol method
1	7·3	6·8	5·3	32	16·5	16·5	12·6
2	7·3	6·6	5·1	33	16·5	16·2	9·3
3	8·3	7·6	6·5	34	16·6	16·2	15·5 (1 h.)
4	9·1	8·5	8·2 (½ h.)	35	16·7	16·5	15·1
5	9·7	9·5	7·1 (1 h.)	36	16·8	15·9	11·7

TABLE I—*contd.*

Soil No.	Percentage of moisture as determined by			Soil No.	Percentage of moisture as determined by		
	Drying over Bunsen burner	Drying in steam oven	Alcohol method		Drying over Bunsen burner	Drying in steam oven	Alcohol method
6	9·7	9·0	7·0	37	17·0	16·7	14·8 (1 h.)
7	10·4	10·2	8·6	38	17·2	17·7	12·8
8	10·5	10·3	9·7 (1 h.)	39	17·4	16·9	14·7
9	11·0	11·0	9·9 (½ h.)	40	17·7	17·6	10·2
10	11·3	10·8	10·9 (½ h.)	41	17·7	17·5	15·2 (1 h.)
11	11·7	10·3	8·3	42	17·7	16·9	15·1
12	11·9	11·8	9·2 (1 h.)	43	18·1	18·3	14·1
13	11·9	11·2	8·1	44	18·3	17·5	12·5
14	12·1	11·3	7·9 (1 h.)	45	18·5	17·5	15·3
15	12·6	12·1	11·3	46	18·9	18·4	7·6
16	12·6	12·6	9·4	47	18·9	18·3	18·4 (1 h.)
17	13·0	12·9	10·3	48	19·7	20·6	13·8
18	13·4	13·1	12·3 (1 h.)	49	20·3	20·1	13·8
19	13·6	13·2	11·5	50	20·4	19·9	14·5
20	14·1	13·3	12·8 (½ h.)	51	20·5	20·9	19·6 (1 h.)
21	14·3	13·8	12·5	52	20·9	20·4	18·7 (1 h.)
22	14·5	14·9	14·1 (1 h.)	53	21·4	20·9	19·6
23	14·5	13·9	13·1 (1 h.)	54	22·4	22·7	12·7
24	15·1	14·5	10·4	55	22·8	22·3	20·5 (1 h.)
25	15·4	14·6	12·8	56	23·8	23·2	18·2
26	15·4	14·8	12·9	57	23·8	24·0	20·0 (1 h.)
27	15·6	16·3	15·0 (1 h.)	58	24·0	23·9	16·6
28	15·7	15·1	15·1 (1 h.)	59	26·9	25·6	18·8
29	15·8	15·8	13·4	60	26·9	25·3	17·9
30	15·9	14·9	13·0	61	27·6	26·3	27·3
31	16·0	15·8	14·5	62	29·4	28·2	19·9

It is evident from the above table that percentage of moisture obtained by drying directly over the Bunsen burner is on the average higher than that obtained by heating overnight in steam oven by 0·5 to 1·0 per cent until the moisture content of the soil exceeded twenty-five per cent when the difference was slightly greater than one per cent. It should be mentioned in this connection that the temperature of the steam oven averages between 98°-99° C. and consequently the percentage of moisture obtained by drying in the oven is lower than the standard percentage obtained by heating overnight at 105°C. So that the actual difference between the true oven-dried percentage and the percentage by drying over Bunsen burner will be much smaller as shown in Table III later on.

With sandy soil precisely the same results as above were observed; but in this case fair agreement of the percentage by the alcohol method with that by the steam oven was obtained until the moisture content of the soil was higher than fourteen to fifteen per cent as will be seen in Table II below.

TABLE II

Showing the comparison between percentages of moisture in Woburn sandy soils as determined by the three different methods

Soil No.	Percentage of moisture as determined by			Soil No.	Percentage of moisture as determined by		
	Drying over Bunsen burner	Drying in steam oven	Alcohol method		Drying over Bunsen burner	Drying in steam oven	Alcohol method
1	5·3	5·4	5·4	7	12·1	13·4	12·1
2	5·8	6·2	5·8	8	13·3	12·9	12·8
3	6·7	7·0	6·4	9	15·0	14·4	12·7
4	8·5	9·3	9·1	10	15·2	14·7	12·9 (½ h.)
5	10·5	10·5	11·2	11	16·0	15·2	14·0 (½ h.)
6	11·7	11·3	11·1	12	18·1	17·4	14·6

Before proceeding further it is intended to discuss briefly the possibilities of error in the Bunsen burner method. This is specially desirable in view of the fact that the method in which the soil is heated directly over the flame may appear drastic at first sight.

From the point of view of heating, the soil can be resolved into four component parts *viz.*, inorganic matter, moisture, organic matter and combined water. The inorganic matter is unaffected by heat in so far as its mass is concerned. The moisture is driven off at 110°-130°C. The organic matter begins to be destroyed at temperatures above 110° C. but the destruction remains nominal until 170°-190°C. Upwards of 250° C. the loss in soil is mainly that of combined water. In the Bunsen burner method, therefore, no burning of organic matter or loss of combined water will take place until all the moisture is driven off, provided the soil is kept stirred and procedures followed as stated in the experimental details. After the soil is apparently dry it is still kept over the flame for half a minute or so to ensure complete loss of moisture. During this time the soil may be heated up to about 200°C. thereby causing some loss of organic matter, but that loss for the reason stated above, is nominal. However, to test this point further, five different soils containing varied amounts of organic matter were subjected to heating over Bunsen burner. These soils were available in the air-dried condition. Consequently they were taken in earthen pots and were exposed to changes in weather for about three weeks. Samples were collected from these pots a day after heavy rainfall. In addition to the Bunsen burner method the

percentage of moisture in these samples was determined by three other methods including the drying in air oven at 105°C. Results which are given in Table III below together with organic matter and clay content of the soils, show that the moisture given by the Bunsen burner method did not vary from the air oven-dry moisture by more than one per cent.

TABLE III

Showing the comparison between percentages of moisture of five different soils as determined by various methods

Soil	Per cent organic matter	Per cent clay	Percentage of moisture as determined by			
			Drying over Bunsen burner	Drying in steam oven	Alcohol method	Drying in air-oven at 105° C.
Argentine	3·4	..	19·3	18·5	15·8	19·0
Cheshire	6·9	15·3	29·0	28·3	24·5	29·2
Sudan	4·3	49·5	46·0	45·3	22·2 35·6 (1 h.)	46·9
Fiji	11·4	31·3	52·1	50·3	40·1	53·0
Fenn	37·0	23·0	73·8	72·0	47·3	74·9

It will thus appear that in soil investigations where a rapid and fairly accurate determination of the moisture content of a soil of any type is wanted the Bunsen burner method as described here will be found suitable. The method, however, is not intended for use in investigations where absolute accuracy in the percentage of moisture is required.

SUMMARY

(1) The possibility of a rapid determination of moisture content of soils by quickly drying the soil over a Bunsen burner was tried and the method as described in this note was found suitable for all types of soil.

(2) The alcohol method for the rapid determination of soil moisture as suggested by Bouyoucos and also by Nitzch was not found suitable for heavy soils.

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STUDIES ON THE ORGANIC NITROGEN OF PADDY SOILS

PART I. DISTRIBUTION OF "HUMIC" AND "NON-HUMIC" NITROGEN IN ORGANIC MATTER

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The practice of maintaining swamp condition in the paddy fields for months during the growth of the crop provides an excellent opportunity of studying the influence of widely-varying and long-continued cultural treatment on the organic matter of the soils. Ever since paddy was grown on them first, these soils have remained alternately dry and water-logged nearly for equal periods, and consequently in such soils, the decomposition of the added plant and animal residues and the further decomposition of the humic matter itself have proceeded under conditions different from those obtaining in normal agricultural soils. It was thought that this difference in treatment might have brought about considerable differences in the nature of the organic matter of the soils and one of the objects of the present investigation is to ascertain whether the organic nitrogen compounds of the paddy soils are different in nature from those occurring in dry cultivated soils (soils not liable to long and frequent water-logging).

Information on the nature of the organic nitrogen compounds of the paddy soils is further required to study the part played by them in the nutrition of paddy. Extensive experiments on the changes of ammonia in paddy soils carried out in this laboratory have shown that although soon after water-logging, the ammonia content of the soils increases, the level drops down shortly so that during the period of the active vegetative growth of the crop, soils contain little or no ammonia. It is believed that at this stage plants assimilate nitrogen in organic forms.

The organic nitrogen of the soils can be fractionated into parts by treating with cold, dilute caustic soda. The scheme of fractionation and the nomenclature employed in this paper are same as those adopted by Hobson and Page [1932] in the study of the organic matter of Rothamsted soils. A part of the soil organic matter, when liberated from its combination with calcium and other metals, becomes readily soluble in cold, dilute alkali. The nitrogen extracted by alkali can be further fractionated by acidifying the solution ; part is precipitated and is called humic nitrogen ; part remains in solution and is called non-humic nitrogen. In the humic fraction, nitrogen occurs as protein or protein-like bodies, while the

non-humic nitrogen includes much simpler and largely soluble compounds as peptides, amino-acids and other protein decomposition products—all of which might have direct values as plant food. Nothing is known about the nature of the nitrogen in the alkali-insoluble fraction.

A number of paddy soils from different parts of India were examined according to the above scheme and the results reported in this paper. Further study on the chemical nature of the non-humic nitrogen compounds of the paddy soils, the part played by them in the nutrition of the crop, their comparison with the corresponding compounds occurring in dry cultivated soils is in progress and will form the subject matters of the future communications.

Experimental

Thirteen unmanured paddy soils, obtained from different parts of India and collected up to a depth of either 0-in.—6-in. or 0-in.—9-in. were air-dried, passed through one-mm. sieve and then submitted to alkali extraction. The methods of alkali extraction and fractionation into humic and non-humic nitrogen were briefly as follows :—

Twenty-gram portions of air-dry soil were shaken with 50 c.c. of two per cent hydrochloric acid in weighed bottles for two hours ; excess of acid was removed and the soil washed several times with distilled water. The bottles were reweighed and the amounts of residual water determined. One hundred c.c. of normal caustic soda and sufficient water to make the total volume of the liquid 200 c.c. were then added to each of the bottles which were shaken for eight hours in an end-over-end shaker. Total nitrogen was determined in an aliquot of the clear alkali extract. For the separation of the humic and non-humic nitrogen from different extracts, 50 c.c. portions of the extracts were acidified with excess of sulphuric acid and the volume made up to 250 c.c. After the precipitates had settled down, 100 c.c. of the clear liquid were taken out for the determination of total nitrogen.

Preliminary experiments carried out to observe the influence of the time of shaking on the extraction of the organic nitrogen showed that the major part of the alkali-soluble nitrogen was extracted after eight hours shaking (Table I).

Table I

Influence of time on extraction of organic nitrogen from soil

Time of shaking	N as per cent of air-dried soils	
	Soil 1	Soil 2
4 hours	0·0462	0·0380
8 „	0·0630	0·0476
16 „	0·0671	0·0490

TABLE II

Nitrogen contents of the different fractions of the organic matter (values expressed as percentage of the air-dried soils)

Soil	N extracted *by direct acid treatment	Alkali-insoluble nitrogen	Humic N	Non-humic N
Karjat, Bombay	0·0012	0·0366	0·0042	0·0175
Sylhet, Assam (B)	0·0018	0·0603	0·0059	0·0420
Sylhet, Assam (C)	0·0060	0·0894	0·0161	0·0455
Rajshahi, Bengal	0·0025	0·0574	0·0112	0·0350
Insein, Burma	0·0014	0·0331	0·0035	0·0175
Coimbatore, Madras	0·0006	0·0476	0·0036	0·0245
Raipur, C. P. (Matasi)	0·0014	0·0438	0·0063	0·0315
Raipur, C. P. (Dorsa)	0·0023	0·0271	0·0014	0·0140
Faridpur, Bengal	0·0014	0·0341	0·0056	0·0210
Dacca, Bengal (E. R.)	0·0013	0·0705	0·0112	0·0420
Dacca, Bengal (N. H.)	0·0018	0·0354	0·0063	0·0385
Rangpur, Bengal	0·0041	0·0461	0·0133	0·0385
Bhagalpur, Bihar	0·0026	0·0697	0·0035	0·0175

* Acid-soluble nitrogen contains, in addition to ammonia and nitrate, little organic nitrogen.

The relative behaviour of the organic matter of the soils can be best brought out by expressing the results as percentages of the total amount of nitrogen in the soils. The results were expressed in this way in Table III.

TABLE III

Nitrogen contents of different fractions of the organic matter (N as per cent of the total soil nitrogen)

Soil	N extracted by direct acid treatment	Alkali-insoluble N	Humic N	Non-humic N
Karjat, Bombay	2	62	7	29
Sylhet, Assam (B)	2	55	5	38
Sylhet, Assam (C)	4	57	10	29
Rajshahi, Bengal	2	54	11	33
Insein, Burma	3	59	6	32
Coimbatore, Madras	1	60	8	31
Raipur, C. P. (Matasi)	2	52	8	38
Raipur, C. P. (Dorsa)	5	61	3	31
Faridpur, Bengal	2	55	9	34
Dacca, Bengal (E. R.)	1	56	9	34
Dacca, Bengal (N. H.)	2	43	8	47
Rangpur, Bengal	4	45	13	38
Bhagalpur, Bihar	3	74	4	23

The major portion of the organic nitrogen of the soils examined is insoluble in cold, dilute alkali, and of the portions soluble in alkali, eighty per cent is non-humic nitrogen. The amount of nitrogen extracted by direct acid treatment is very small, representing only three per cent of the total soil nitrogen. The very low value for humic nitrogen indicates that these soils are very poor in humus, obviously due to rapid oxidation of organic matter in soils under Indian climate. The general distribution of nitrogen in the organic matter of paddy soils is approximately as follows :—

N extracted by direct acid treatment	3 per cent
Alkali-insoluble nitrogen	56 ,,
Non-humic nitrogen	33 ,,
Humic nitrogen	8 ,,

An examination of the figures for the alkali-insoluble and the non-humic nitrogen, in Table III, will bring out a very remarkable fact. Barring the last three soils which showed some discrepancy, the proportions of the alkali-insoluble and the non-humic nitrogen in rest of the soils are nearly equal, inspite of the fact that these soils were obtained from localities, widely apart and under different climatic conditions. It is thus apparent that the organic matter of the paddy soils has acquired some uniform properties, presumably as a result of the similar treatments applied to these soils.

Extraction of nitrogen by baryta and sodium carbonate

Twenty-gram portions of soil after preliminary acid treatment, were extracted with *N*/2 barium hydroxide and *N*/5 sodium carbonate for eight hours, the procedure being identical with that described before. The results show that much less nitrogen is extracted by these solvents than by caustic soda.

Table IV

Extraction of nitrogen by baryta and sodium carbonate

(*Results are expressed as percentage of total soil-nitrogen*)

Solve	Assam soil			Rajshahi soil			Dacca soil		
	N extracted	Humic N	Non-humic N	N extracted	Humic N	Non-humic N	N extracted	Humic N	Non-humic N
N/2 NaOH . . .	39	10	29	44	11	33	43	9	34
N/2 $Ba(OH)_2$ . . .	10	1	9	6	0	6	15	1	14
N/5 Na_2Co_3 . . .	14	7	7	6	3	3	23	9	14

Rate of extraction of humic and non-humic nitrogen from soil

The manner how non-humic nitrogen compounds exist in soils is a matter of much importance. Hobson and Page [1932] suggested that these compounds

remain " incorporated in the humic-clay gel so that for the most part they go into solution only in solvents which dissolve the humic matter, and in proportion to the amount so dissolved ". In order to test this hypothesis further, and also to have some information about the manner of association between the non-humic and humic compounds of the paddy soils, two soils were extracted with alkali for varying lengths of time and the distribution of non-humic and humic nitrogen in different extracts studied. The results are as follows :—

TABLE V

Rate of extraction of humic and non-humic nitrogen from soil

(*N as per cent of total nitrogen of soil*)

	Time of extraction	Nitrogen in extract	Non-humic N	Humic N
Soil 1	1 hour	28	22	6
	3 „	33	25	8
	5 „	37	27	10
	8 „	41	29	12
	16 „	43	31	12
Soil 2	1 „	33	26	7
	3 „	34	26	8
	5 „	37	26	11
	8 „	45	33	12
	16 „	46	33	13

The results obtained with soil 1 are compatible with the suggestion of Hobson and Page. But in soil 2, the non-humic nitrogen was extracted in two instalments —nitrogen in the first instalment which came after one hour's shaking was probably linked with the humic matter, since they came into solution together. But the second instalment which was extracted after eight hours' shaking must have been associated in a different way ; for it was obtained when humic matter practically ceased to dissolve.

SUMMARY

Thirteen unmanured paddy soils from different parts of India were examined with regard to the solubility of the organic nitrogen in cold, dilute alkali and the distribution of humic and non-humic nitrogen in the alkali extracts.

It has been found that the average distribution of nitrogen in the organic matter of these soils is as follows : Nitrogen extracted by direct acid treatment—three per cent, alkali-insoluble nitrogen—fifty-six per cent, non-humic nitrogen—thirty-three per cent, humic nitrogen—eight per cent.

Caustic soda extracted much greater amount of nitrogen from the soils than baryta or sodium carbonate.

Evidence was obtained that the non-humic nitrogen compounds may occur in the soil in some manner other than being incorporated in the humic-clay gel.

The authors' thanks are due to Professor J. C. Ghosh for his interest in the progress of the work. Thanks are also due to the Imperial Council of Agricultural Research for maintaining the salary of one of them (P. K. De).

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THE INHERITANCE OF SEED-CHARACTERS IN GRAM (*CICER ARIETINUM* L.)

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(With Plates LX and LXI)

CONTENTS

	PAGE
I. INTRODUCTION	1087
II. DESCRIPTION OF PARENTS AND F_1	1089
III. RESULTS OF THE F_2 GENERATIONS	1089
IV. THEORY AND DISCUSSION OF RESULTS	1093
V. SUMMARY	1104
REFERENCES	1104

I. INTRODUCTION

The inheritance of characters in gram has been under study at the Botanical Sub-Station, Karnal, since the year 1931. The results of these investigations, dealing exclusively with the floral characters, have recently been published [Abdur Rahman Khan and Akhtar, A. R., 1934]. During the course of this work it became evident that the segregation of petal colour, reported in the previous publication, should be continued with the study of the inheritance of seed-characters.

(*1*) *Seed-colour.*—This is an important character in gram as it determines its price to a certain extent. From the market point of view, uniform yellowish brown colour fetches a higher price than dark and reddish brown. Some colours such as yellowish brown, reddish brown and dark reddish brown exhibit a certain amount of variation even among the seeds of a single plant. This variability is largely due to poor development and defective setting of seeds. Seed-colour,

again, is somewhat altered with age. Yellow or reddish colours become darker even after a few months ; brightness of colour fades and seeds appear dull. The following points have, therefore, to be borne in mind while recording observations on seed-colours :—

(1) Observations should be taken when seeds are quite fresh.

(2) Colour should be judged in mass. The colour of individual seeds always shows slight differences. Seeds can best be seen when placed in a Petri dish which facilitates noting of general colour.

(3) Shrivelled and poorly developed seeds should be left out and observations be confined to sound seeds only. In many cases improperly developed seeds look greenish and the real colour is not seen at all.

(2) *Seed-shape.*—This character, too, is somewhat variable. Round shape shows a good deal of variation in certain types. Seeds of type 11, for instance, which are perfectly round always have a few slightly irregular seeds. The variation is exclusively confined to seeds which are less healthy and shrivelled. The larger the number of poorly developed seeds, the greater is the proportion of slightly irregular seeds. Irregular shape is, however, much less affected by improper development. As in colour, observations should be taken only on healthy and well-developed seeds.

(3) *Seed-surface.*—In gram, seed-surface may be puckered, smooth, granulated or rough. These surfaces are clearly marked from one another but the intermediate grades such as slightly granulated, slightly puckered, etc., are sometimes difficult to distinguish but can be differentiated from smooth. The smooth and granulated surfaces are best distinguished under a lens. The granulated surface will show many small granulations as pustules on the seed-coat. The rough seed-coat is somewhat like sand-paper to the touch.

(4) *Black dots.*—When seeds of certain types are examined under a lens, it is seen that there are minute black dots on the seed-coat. In such cases, a number of seeds have to be examined very closely to detect the presence of these dots.

The above-mentioned characters have been studied in the following crosses and the results obtained are described in this paper :—

(1) T. 12 × T. 11.
(2) T. 52 × T. 11.
(3) T. 21 × T. 11.

(×1½) Parent T. 12. (×3)

(×1½) Parent T. 52. (×3)

(×1½) (×3)

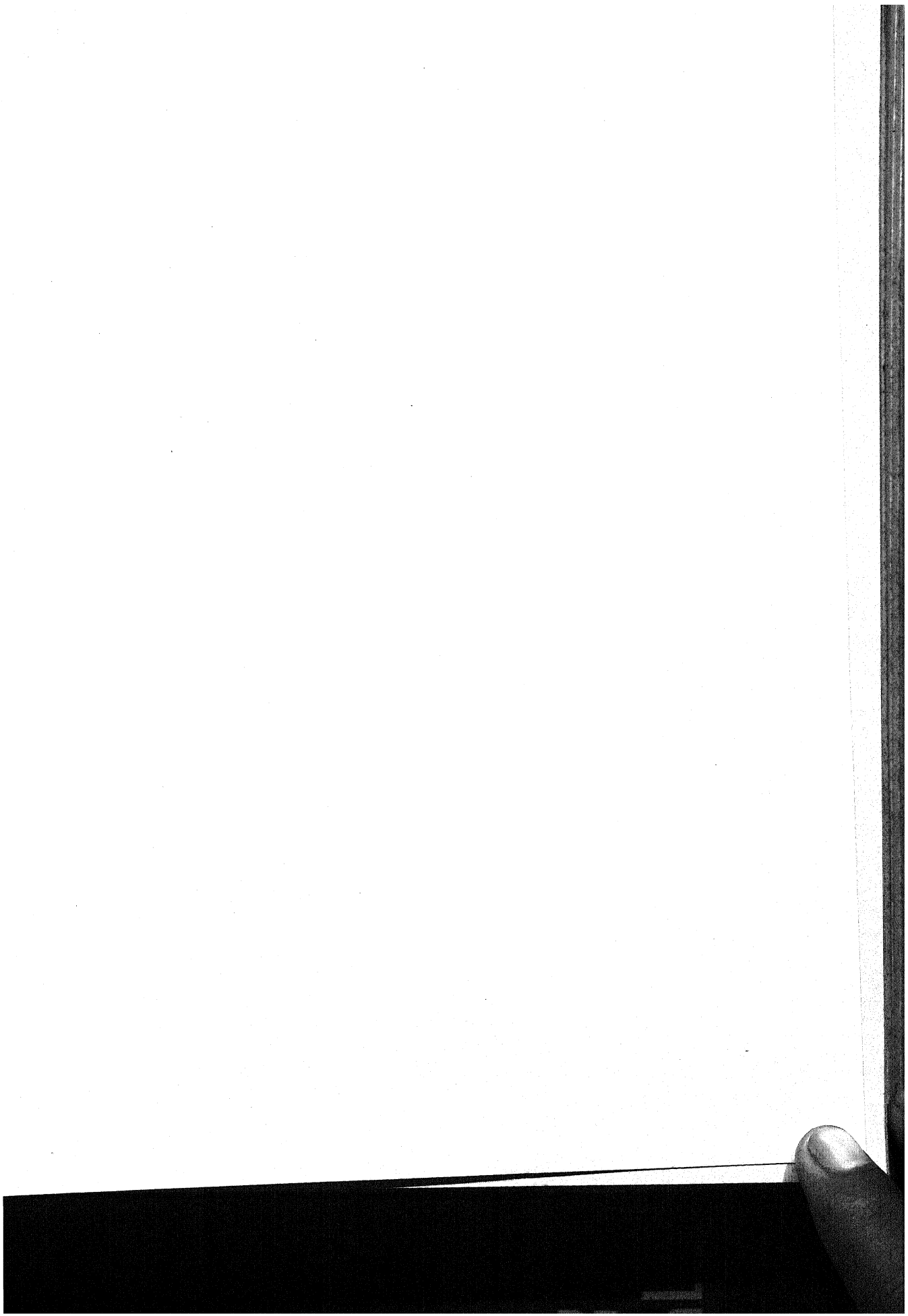
Parent T. 11.

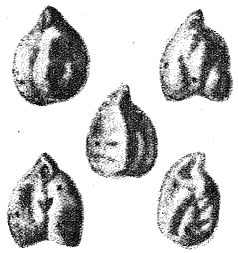
(×1½) (×3)

T. 52×T. 11. F1 Yellowish brown.

(×1½) (×3)

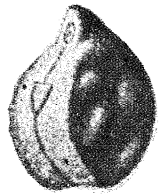
T. 12×T. 11. F1 Reddish-brown.



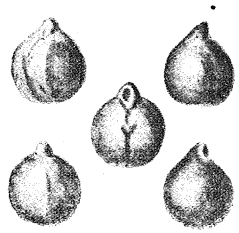


Parent
T. 21.

(×1½)



(×3)



(×1½)

(×3)

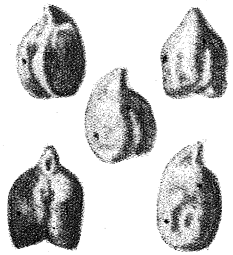
Parent
T. 11.

(×1½)

(×3)

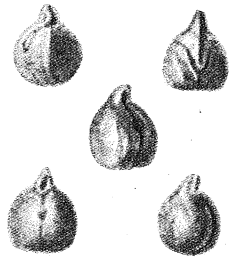
T. 21 x T. 11.
F_1
Dark–reddish–brown.

F_2



Dark–reddish–brown.
(×1½)

Yellowish–brown.
(×1½)



Bluish–brown.
(×1½)

PLATE LXI

II. DESCRIPTION OF THE PARENTS.

(From Shaw, F. J. F. and Khan, A. R., 1931) and F_1

Parents and F_1	Flower colour	Seed-colour	Seed-shape	Seed-surface	Presence or absence of black dots
Type 11 . .	White	Bluish brown .	Roun	Smooth .	Absent
„ 12 . .	„	Reddish brown	Irregular	„ .	Absent*
„ 21 . .	Pink .	Dark reddish brown.	„	Smooth (slightly granulated).	Present
„ 52 . .	„ .	Yellowish brown	„	„	„
12×11 (F_1) .	White	Yellowish to reddish brown.	„	Smooth .	Absent
52×11 (F_1) .	Pink .	Yellowish brown	„	As in T. 52 .	Present
21×11 (F_1) .	„ .	Dark reddish brown lighter than T. 21.	„	As in T. 21 .	„

The seed-characters of the parents and F_1 are illustrated in Plates LX and LXI.

* A very few faint dots are sometimes present on seeds of T. 12 but as they are hardly visible to the naked eye they have been treated as absent.

III. RESULTS OF THE F_2 GENERATIONS

Cross No. 1 (T. 12×T. 11)

The following segregation was observed in the F_2 :—

Phenotypes	Frequencies		Deviation	Probable error
	Observed	Expected on 3 : 1 ratio		
Seed, yellowish to reddish brown, irregular as in T. 12.	22	22·5	0·5	1·59
Seed, bluish brown, round as in T. 11 .	8	7·5	0·5	
Total .	30			

The fit is good.

Considering the segregation, character by character, it is evident that reddish brown colour and irregular shape are dominant over bluish brown colour and round shape respectively and each case shows a unit factor difference.

Association of characters.—It has been observed in this cross that the reddish brown colour and irregular shape, and similarly bluish brown colour and round shape, go together. This shows that these characters are probably linked together and the F_3 results, as will be seen later lend support to this assumption.

Cross No. 2 (T. 52 × T. 11)

Segregations in the F_2 :—

Phenotypes	Frequencies		Deviation	Probable error
	Observed	Expected on 3 : 1 ratio		
Seed, yellowish brown, irregular, slightly granulated as in T. 52.	39	41·25	2·25	2·16
Seed, bluish brown, round and smooth as in T. 11.	16	13·75	2·25	
Total .	55			

The fit is good.

Presence of black dots, on seed-coat in F_2

Phenotypes	Frequencies		Deviation	Probable error
	Observed	Expected on 15 : 1 ratio		
Black dots present	51	51·56	0·56	1·24
„ „ absent	4	3·44		
Total .	55			

The fit is good.

It is evident from the above results that yellowish brown colour, irregular shape, slightly granulated surface and presence of black dots are dominant over bluish brown colour, round shape, smooth surface and absence of black dots respectively. Each one of these characters, excepting the presence of black dots which seems to be determined by two factors, shows a single factor difference.

In the F_2, the following results were obtained with respect to flower-colour and seed-colour :—

Flower-colour	Pink	Blue	White	
Seed-colour	Yellowish brown.	Bluish brown	Yellowish brown.	Bluish brown
Seed-shape	Irregular	Round	Irregular	Round
Seed-surface	Slightly granulated.	Smooth	Slightly granulated.	Smooth
Black dots	Present prominently.	Present faintly.	Present faintly.	Absent
Observed frequencies	31	12	8	4
Expected on 9 : 3 : 3 : 1 ratio.	30·94	10·31	10·31	3·44

$\chi^2 = 0{\cdot}8857$, when $\chi^2 = 1$, $P = 0{\cdot}801253$. The fit is excellent.

This table also explains the segregation of black dots on the basis of 15 : 1 ratio as mentioned before. The black dots are many and prominent when the flower is pink. They are few and faint when the flower is blue and seed bluish brown in colour or when flower is white and seeds are yellowish brown. This appears to be due to the cumulative effect of two factors—each one producing faint dots. These faint dots are practically absent when seeds have a reddish or greenish tinge and in such cases only a dot or two is revealed by a close examination under a lens. When, however, seeds are badly developed and shrivelled the dots are not seen at all. This may be due to imperfect development of the seeds.

Cross No. III (T. 21 × T. 11)

In the F_2 generation the following segregation was observed :—

(*I*) *Seed-colour*

Phenotypes	Frequencies		(O—C)	$\frac{(O-C)^2}{c}$
	Observed	Expected on 9 : 3 : 4 ratio		
Dark reddish brown	33	29·81	3·19	0·34
Yellowish brown	10	9·94	0·06	0·0005
Bluish brown	10	13·25	3·25	0·79
Total	53	..	$\chi^2 = 1{\cdot}13$ $P = 0{\cdot}573$	

The fit is good.

(2) *Seed-shape and —surface*

Phenotypes	Frequencies		Dev.	P. E.
	Observed	Expected on 3 : 1 ratio		
Irregular and slightly granulated .	43	39·75	3·25	2·12
Round and smooth	10	13·25		
Total .	53	..	..	..

The fit is good.

(3) *Black dots*

Phenotypes	Frequencies		Dev.	P. E.
	Observed	Expected on 15 : 1 ratio		
Black dots present	50	49·69	0·69	1·15
Black dots absent	3	3·31		
Total .	53	..	..	..

The fit is good.

Association of seed-characters

The following table illustrates this :—

Seed-colour	Shape	Surface	Black dots	Obs.
Dark reddish brown . .	Irregular .	Slightly granulated.	Present .	33
Yellowish brown . . .	Do. .	Do. .	Do. .	10
Bluish brown . . .	Round .	Smooth .	Present .	7
			Absent .	3

Seed-characters in relation to flower-colours

As seen in cross No. II seed colour, etc., show a definite relation with flower-colour as is evident from the following table :—

Table showing the relation of flower-colour with the seed-characters

Flower-colour	Seed-colour	Seed-shape	seed-surface	Black dots	Obs.
Pink . .	Dark reddish brown.	Irregular	Slightly granulated.	Present prominently.	20
,, . .	Yellowish brown	,, .	,, .	,, .	7
White . .	Dark reddish brown.	,, .	,, .	Present faintly.	13
,, . .	Yellowish Brown	,, .	,, .	,, .	3
,, . .	Bluish brown .	Round .	Smooth .	Absent .	3
Blue . .	,, .	,, .	,, .	Present faintly.	7
				Total .	53

The inheritance of black dots on seed appears to be the same as observed in cross II.

IV. Theory and discussions of the results with reference to the F_3 generation

A=a factor for bluish brown seed-colour, round shape, smooth surface. It has no action on flower-colour.

R=a factor that modifies the action of **A** turning bluish brown colour to reddish brown and round to irregular seed.

B=a factor which operates in the same way as **A** but in addition produces blue flower and faint black dots on seeds.

P=a factor that modifies the action of **B** turning blue flower to pink. It reacts with **A** as well as with **B**, converting bluish brown seeds to yellowish brown, round to irregular and smooth to slightly granulated. It produces faint black dots on seed when present alone, but reacts with **B** to intensify black dots on the seed-coat.

D=a factor that modifies the action of **P** turning yellowish brown seed-colour to dark reddish brown.

On the assumption of the above theory, the constitutions of the parental types are as given below :—

T. 11 = **AArrbbppdd**
T. 12 = **AARRbbppdd**
T. 52 = **AArrBBPPdd**
T. 21 = **AArrBBPPDD**

NOTE.—By supposing that the flower-colour, factor **P** produces yellowish brown seed- colour independently it is not necessary to have factor **A** in the constitution of the types. But the factor **P** has been found to be present in T. 1, T. 2 and T. 9 [Khan and Akhtar, 1934] and the seed-colour of these types is white with reddish tinge and not yellowish brown. Factor **P** has, therefore, been supposed to modify bluish brown colour that is produced by factor **A**.

Cross I (T. 12 × T. 11)

In this cross the factor **R** segregates and on recombination gives a mono-hybrid ratio as realised in the F_2 generation.

The results of the F_3 generation, as will be seen below, have confirmed the above theory. Sixteen cultures were grown in the F_3 generation and these behaved as follows :—

Number and nature of F_2 parent	Behaviour in F_3 generation	Constitution of F_2 genotype	Frequencies	
			Observed	Expected
11 Reddish brown irregular	Pure	**AARR**	5	3·66
	Segregating like F_2	**AARr**	6	7·34
5 Bluish brown round	Pure	**AArr**	5	5·00

The six splitting cultures showed the following segregation :—

F_3 cultures number	Reddish brown irregular	Bluish brown round	Total
1—41	28	10	38
1—42	28	5	33
3—44	27	10	37
3—48	28	12	40
4—41	28	9	37
3—47	28	10	38
Total observed	167	56	223
Expected on 3 : 1 ratio	167·25	55·75	..
Deviation	0·25	0·25	..

The fit is good.

During the course of observations some F_3 cultures showed a certain amount of variation from the parental seed-colour. The seed-colour was found to vary from deep reddish brown to light reddish brown. On breeding a few F_3 plants from each of the extreme classes, the F_4 progenies showed uniformity as regards seed-colour, thereby indicating that the apparent variations in F_3 were but fluctuations due to environments.

Cross II (T. 52 × T. 11)

The constitutions of the parents and F_1 :—

Parent type 52 = **AABBPPrr**
,, 11 = **AAbbpprr**
F_1 = **AABbPprr**

The factors **B** and **P** alone segregate and give the following results in F_2 generation.

	9 ABP	3 ABp	3 AbP	1 Abp
Flower-colour	Pink	Blue	White	White
Seed-colour	Yellowish brown.	Bluish brown	Yellowish brown.	Bluish brown
Seed-shape	Irregular	Round	Irregular	Round
Seed-surface	Slightly granulated.	Smooth	Slightly granulated.	Smooth
Black dots	Present prominently.	Present lightly.	Present lightly.	Absent

The results obtained in the F_2 generation are in perfect agreement with this theory (page 1091) and were subsequently confirmed in F_3 as shown below.

Twenty-nine F_2 plants representing the various phenotypes were grown in F_3 generation and their behaviour with regard to seed-characters is given in the following table :—

No. and nature of F_2 plants	Behaviour in F_3	Constitution of F_2 genotypes	Frequencies	
			Observed	Expected
23 Yellowish brown irregular, slightly granulated.	Pure	**AABBPP**, **AABbPP**, **AAbbPP**	11	7·7
	3 yel. br. : 1 blu. br.	**AABBPp**, **AAbbPp**, **AABbPp**	12	15·3
6 Bluish brown, round smooth.	Pure	**AABBpp**, **AABbpp**, **AAbbpp**	6	6

The segregation of the twelve splitting cultures is as follows :—

F_3 cultures number	Seeds yellowish brown, irregular, slightly granulated	Seeds bluish brown, round, smooth	Total
4—34	21	9	30
4—37	15	16	31
4—40	17	12	29
3—2	28	9	37
3—27	25	6	31
3—24	25	11	36
4—15	24	12	36
4—39	14	11	25
3—9	14	5	19
3—32	25	13	38
3—33	29	9	38
4—14	26	12	38
Total observed . . .	263	125	388
Expected	291	97	..
Deviation	28	28	..
	Probable error . .	5·73	

The fit is bad.

On examining the segregation of individual F_2 plants, given in the above table, it will be seen that this discrepancy is mainly confined to three F_3 cultures, *viz.*, 4—37, 4—39 and 4—40, while all the rest give a fairly good fit. Again it must be mentioned that some plants having pink flowers and evidently yellowish brown seeds, had to be rejected as the seeds from these were poorly developed and shrivelled. If these are added the deviation is reduced.

The behaviour of F_3 cultures in seed-colour is better understood when considered with flower-colour. This is shown below :—

The behaviour of F_3 cultures in relation to seed-colour and flower-colour

Number and nature of parents	Behaviour in F_3			Constitution of F_2 genotypes	Frequencies		Group
	Flower-colour	Seed-colour	Black dots on seed coat		Observed	Expected	
18 .	Pure pink .	Pure yellowish brown	Pure for prominent dots	**AABBPP** .	2	2	I
Flower pink and seed yellowish brown, etc.	3 pink : 1 white .	Do. .	3 Prom. : 1 Faint .	**AABbPP** .	8	4	II
	3 pink : 1 blue .	3 yell. br. : 1 blu. br.	3 Prom. : 1 Faint .	**AABBPp** .	3	4	III
	9 pink : 3 blue : 4 white.	12 yell. br. : 4 blu. br.	Like F_2 . .	**AABbPp** .	5	8	IV
5							
Flower white and seed yellowish brown, etc.	Pure white .	Pure yell. br. .	Pure for faint dots	**AAbbPP** .	1	1·7	V
	Do. . .	3 yell. br. : 1 blu. br.	3 Faint : 1 no dots .	**AAbbPp**	4	3·3	VI
2							
Flower white and seed bluish brown, etc.	Pure white .	Pure bluish brown .	Pure for no dots .	**AAbbpp** .	2	2	VII
	Do. .	Do. .	Do. .				
4							
Flower blue and seed bluish brown, etc.	Pure blue . .	Pure bluish br. .	Pure for faint dots .	**AABBpp** .	0	1·3	VIII
	3 Blue : 1 white .	Do. .	3 Faint : 1 no dots .	**AABbpp** .	4	2·7	IX

The segregation in the 8 F_3 cultures of group II was :—

	Flower pink (black dots on seed prominent)	White (black dots on seed faint)	Total
Total observed	208	74	282
Expected on 3 : 1 ratio	211·5	70·5	(P. E. =4·90)

The fit is good.

Segregations in group III :—

Culture No.	Flower pink, seed yellowish brown, black dots prominent	Blue, bluish brown, etc., black dots faint	Total
4—34	21	9	30
4—37	15	16	31
4—40	17	12	29
Total observed	53	37	90
Expected on 3 : 1 ratio	67·5	22·5	..
Deviation	15·5	P. E. =2·76	

The fit is not good due to discrepancy in cultures 4—37 and 4—40 owing to deficiency in pink flowered plants as explained before (page 1096).

Segregation in group IV :—

Culture No.	Flower pink		Flower white		Flower blue
	Seed yellowish brown, etc., dots present	Yellowish brown, etc., dots present	Bluish brown dots absent	Bluish brown, etc., dots present	
3—2	19	9	2	7	
3—27	19	6	2	4	
3—24	19	6	0	11	
4—15	18	6	4	8	
4—39	11	3	2	9	
Total observed	86	30	10	39	
	116		49		=165
Expected on 9 : 3 : 1 : 3 ratio.	92·82	30·93	10·31	30·93	
	123·75		41·25 (P.E. 3·37)		

P=0·4422, χ^2=2·72.

Considering dots independently we get :—

	Dots present	Dots absent
Total observed	155	10
Expected on 15 : 1 ratio	154·59	10·31

The fit is good.

There is an excess in blue flowered plants and deficiency in pink. This may be due to the rejection of some pink flowered plants that had poorly developed seeds and so could not be taken into account in the classification of seed-colour and also to the small population in the different cultures.

Segregation in group VI (white flowered).

Culture No.	Yellowish brown, irregular, etc., black dots faint	Bluish brown, round, etc., black dots absent
3—9	14	5
3—32	25	13
3—33	29	9
4—14	26	12
Total observed	94	39
Expected on 3 : 1 ratio	99·75	33·25
Probable error	3·27	

Similarly in group IX no black dots could be seen on the bluish brown seeds having white flowers.

Cross III (T. 21 × T. 11)

Parent Type 21 = **AABBPPDD**

Parent Type 11 = **AAbbppdd**

In the F_2 generation factors **B**, **P** and **D** segregate and give the following results :—

——	Flower colour	Seed colour	Frequeney	
			Observed	Expected
27—**ABPD**	Pink	Dark reddish brown	20	22·41
9—**ABPd**	,,	Yellowish brown	7	7·47
9—**AbPD**	White	Dark reddish brown	13	7·47
9—**ABpD**	Blue	Bluish brown	7	9·96
3—**ABpd**	,,	,, ,,		
3—**AbPd**	White	Yellowish brown	3	2·49
3—**AbpD**	,,	Bluish brown	3	3·32
1—**Abpd**	,,	,, ,,		
Total	..	..	53	..

The expected frequency is based on the ratio of 27 : 9 : 9 : 12 : 3 : 4.

From the above table we get the following segregation in F_2 :—

1. Seed-colour

Phenotypes	Seed-colour	Frequencies	
		Observed	Expected on 36 : 12 : 16 ratio
27—ABPD	Dark reddish brown	33	29·81
9—AbPD	,, ,, ,,		
9—ABPd	Yellowish brown .	10	9·94
3—AbPd	,, ,, .		
9—ABpD	Bluish brown .	10	13·25
3—ABpd	,, ,, .		
3—AbpD	,, ,, .		
1—Abpd	,, ,, .		

The fit is good as mentioned before (page 1091).

2. Seed-shape, surface, and presence of black dots being linked with seed-colour will all behave similarly as shown below :—

Phenotypes	Seed-colour	Shape, surface black dots	Frequencies	
			Obs.	Expected on 48 : 16 ratio
27—ABPD . . .	Dark reddish	Irregular, slightly		
9—AbPD.	brown.	granulated.	43	39·75
9—ABPd . . .	Yellowish	Having black dots .		
3—AbPd.	brown.			
9—ABpD . . .	Bluish brown	Round, smooth having black dots.	7	13·25
3—ABpd . . .			10	
3—AbpD . . .		Round, smooth black dots absent.	3	
1—Abpd . . .				
		Total .	53	

F_3 results

Twenty-eight cultures were grown in F_3. Their behaviour with regard to seed-characters is given below :—

Number and nature of F_2 parents	Behaviour in F_3	Constitution of F_2 genotype	Frequencies	
			Observed	Expected
16 Seed dark brown, irregular, etc.	Pure dark brown irregular, etc.	AABBDDPP AABbDDPP AAbbDDPP	3	1·77
	3 dark brown, to 1 yellow brown (irregular, etc.).	AABBDdPP AABbDdPP AAbbDdPP	6	3·55
	3 dark brown, irregular, etc. 1 bluish brown, round, etc.	AABBDDPp AABbDDPp AAbbDDPp	3	3·55
	9 dark brown, irregular, etc. 3 yellowish brown, irregular, etc. 4 bluish brown, round, etc.	AABBDdPp AABbDdPp AAbbDdPp	4	7·11
5 Seed yellowish brown, irregular, etc.	Pure yellow brown, irregular, etc.	AABBddPP AABbddPP AAbbddPP	1	1·66
	3 yellow brown, irregular, etc. 1 bluish brown, round, etc.	AABBddPp AABbddPp AAbbddPp	4	3·33
7 Seed bluish brown, round, etc.	Pure	AABBDDpp	7	7
	bluish, brown, round, etc.	AABbDDpp AABBDdpp AABbDdpp AABBddpp AABbddpp AAbbDDpp AAbbDdpp AAbbddpp		

The details of F_3 behaviour tabulated above are given hereunder.

Sixteen cultures from dark reddish brown, irregular, slightly granulated seed with black dots were grown in F_3. Out of these three bred true and six segregated into dark reddish brown and yellowish brown seeds as follows :—

——	Dark reddish brown	Yellowish brown	Total
Observed	152	52	204
Expected 3 : 1 . . .	153	51	..

Three cultures segregated into dark reddish brown to bluish brown seeds as follows :—

	Dark reddish brown	Bluish brown	Total
Total observed . . .	52	20	72
Expected 3 : 1 . . .	54	18	..

The remaining four cultures segregated like F_2 and gave the following results :—

	Dark reddish brown	Yellowish brown	Bluish brown	Total
Total observed . . .	75	21	33	129
Expected 36 : 12 : 16 (9 : 3 : 4)	72·56	24·19	32·25	..

Out of the five cultures having yellowish brown, irregular, slightly granulated seeds with black dots, one culture bred true. The remaining four segregated as follows :—

	Yellowish brown	Bluish brown	Total
Total observed . . .	84	28	112
Expected on 3 : 1 . . .	84	28	..

All the seven cultures having bluish brown, round, smooth and very small seeds (one without black dots and six having black dots), bred true as per expectation.

F_3 behaviour of flower and seed colours

Number and nature of F_2 parent	Behaviour in F_3		Constitution of F_2 genotypes	Frequencie s	
	Flower-colour	Seed-colour		Observed	Expected
12 Flower—pink. Seeds—dark brown.	Pure pink 3 pink : 1 white Pure pink 3 pink : 1 white Do. 9 pink : 3 blue : 4 white Do. 3 pink : 1 blue 3 pink : 1 white	Pure dark brown Do. 3 dr. br. : 1 yel. br. Do. 3 dr. br. : 1 blu. br. Do. 9 dr. br. : 3 yel. br. : 4 blu. br. Do. Do.	AABBDDPP. AABbDDPP. AABBDdPP. AABbDdPP. AABBDDPp AABbDDPp. AABbDdPp. AABBDdPp. AAbbDdPp.	0 2 1 3 0 2 1 2 1	0·44 0·88 0·44 1·76 0·88 1·76 1·76 3·52 1·76
4 Flower—white. Seed—dark brown.	Pure white Do. Do.	Pure dark brown 3 dr. br. : 1 yel. br. 3 dr. br. : 1 blu. br.	AAbbDDPP. AAbbDdPP. AAbbDDPp.	1 2 1	0·44 0·88 0·88
4 Flower—pink. Seed—yellowish brown.	Pure pink 3 pink : 1 white 3 pink : 1 blue 9 pink : 3 blue : 4 white	Pure yellowish brown Do. 3 yel. br. : 1 blu. br. Do.	AABBddPP. AABbddPP. AABBddPp. AABbddPp.	0 1 2 1	0·44 0·88 0·88 1·76
1 Flower—white. Seed—yellowish brown.	Pure white Do.	Pure yellowish brown 3 yel. br. : 1 blu. br.	AAbbddPP. AAbbddPp.	0 1	0.44 0.88
1 Flower—white. Seed—bluish brown.	Pure white Do.	Pure bluish brown	AAbbDDpp. AAbbDdpp. AAbbddpp.	1	1·76
6 Flower—white. Seed—bluish brown.	Pure blue Do. 3 blue : 1 white	Pure bluish brown .... Do.	AABBDDpp. AABBDdpp. AABBddpp. AABbDDpp. AABbDdpp. AABbddpp.	1 3	1·76 3·42

It is evident from these results that the seed-characters and flower-colour are perfectly linked as observed in F_2 generation.

The behaviour of black dots in the F_3 generation of this cross was similar to that described in Cross II (T. 52 × T. 11) and so has not been repeated.

NOTE :—Large populations of all the crosses were grown in the F_2 generation and F_3 cultures but unfortunately the gram plants were damaged much by *Rhizoctonia* at Karnal and so extensive observations could not be recorded.

The seed-size was studied by eye judgment in all the crosses. The seeds of T. 11 may be called as very small and those of other types as comparatively larger. Very small size went together with bluish brown seeds as in T. 11 and larger seeds went with the seed-colour of other types. The results regarding seed-size have not been presented because the populations of the crosses were not big enough to ascertain definitely whether the seed-size which is expected to be a quantitative character is really behaving as mentioned above.

The authors desire to acknowledge the help rendered by Mr. Habibur Rahman Khan, Fieldman, during the course of these investigations.

V. SUMMARY

The inheritance of colour, shape and surface of seed, has been studied in the three crosses between Pusa gram types 11, 12, 52 and 21. The results of segregations have been given and discussed. The following facts have been noted :—

(1) The bluish brown seed-colour as in type 11 is dependent on a single factor **A** or **B**.

(2) The reddish brown seed-colour of type 12 is produced by the interaction of factors **A** and **R**.

(3) The factor **P** modifies the action of **A** or **B** turning bluish brown seed-colour to yellowish brown as in type 52.

(4) The dark reddish brown colour of seed as in type 21 is determined by the interaction of the factors **A**, **P** and **D** or **B**, **P** and **D**.

(5) In all the three crosses, the bluish brown seed-colour of the common parent, type 11, behaves as a recessive to reddish brown, yellowish brown and dark reddish brown seed-colours of types 12, 52 and 21 respectively.

It is shown that the colour, shape and surface of seed are all associated.

Group I.—The yellowish brown and dark reddish brown colours are linked with irregular shape and slightly granulated surface.

Group II.—The bluish brown colour is linked with round shape and smooth surface.

Besides the above associations it is clear that the flower-colour is also linked with these seed-characters—pink flower is associated with group I with prominent black dots, and blue flower with group II having faint black dots.

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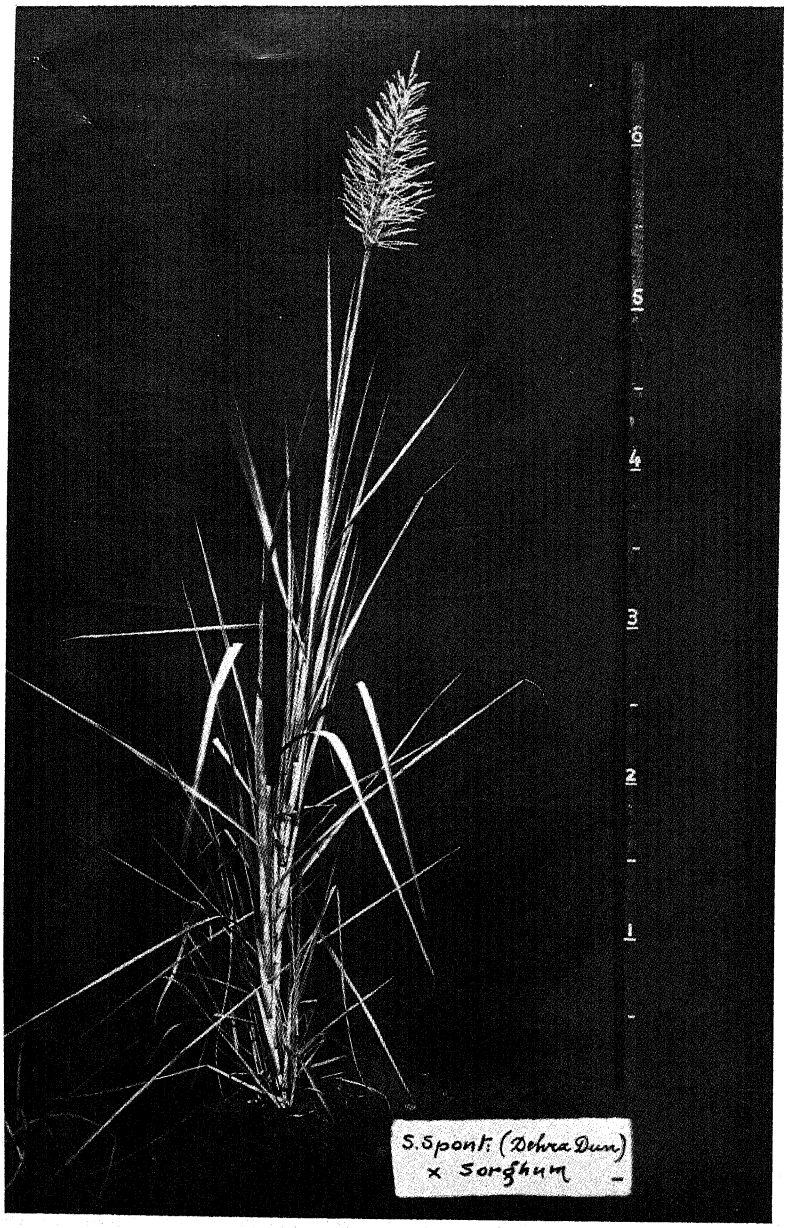

S. spontaneum (Dehra Dun) × *Sorghum Durra*.

A PRELIMINARY NOTE ON A NEW SACCHARUM × SORGHUM HYBRID

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(With plates LXII and LXIII)

In sugarcane-breeding it has been the custom to use as female parents canes that are pollen-sterile. Such plants are also generally complex hybrids. Thus in the successful intergeneric hybrids evolved at Coimbatore [Venkatraman and Thomas, 1930] the female parent used, P. O. J. 2725, was a noble cane from Java with a complex heredity. It has further about 106 chromosomes.

With a view to understanding the genetic relationship between the genus *Saccharum* and *Sorghum*, a number of crosses were made in 1934-1935 in which the female parents were simpler forms of *Saccharum*. Amongst such parents employed was the wild species, *Saccharum spontaneum* L. which is being increasingly used in Coimbatore for the breeding of disease and drought-resisting sugarcanes. A number of chromosomal forms are found in this species [Janaki Ammal, 1936] and the hybrid reported is a cross between a form from Dehra Dun ($2n=56$) and *Sorghum durra* (*Periamanjal* of Coimbatore), the same parent used in the intergeneric cross P. O. J. 2725 × *Sorghum*. In *Sorghum durra* the chromosome number $2n=20$.

As the *S. spontaneum* used was nearly 100 per cent pollen-fertile, pollination with *Sorghum* was effected after emasculation of spikelets. The arrows were bagged after dusting with *Sorghum* pollen. Thirteen seedlings were produced, all of which are now in field. In appearance they resemble *S. spontaneum* more than *Sorghum* (Plate LXII). The plants arrowed five months after germination, *i.e.* four months before the usual time of flowering in *Saccharum*. This early flowering habit is apparently inherited from *Sorghum*. It was however found to be completely pollen-sterile.

Plate LXIII shows the nature of the spikelet of the parents and hybrid.

S. spontaneum differs from *S. officinarum* in having a rudimentary IV glume. This IV glume, which is also present in *Sorghum*, is awned in this genus. The presence of this awned IV glume in the hybrid as well as the callus hairs inherited from *Saccharum* makes the validity of this new intergeneric cross unquestionable. The number of chromosomes in root tip of the hybrid is thirty-eight which is the sum of the haploid numbers of the Dehra Dun *S. spontaneum* and *Sorghum*. A more detailed study of its cytology will appear elsewhere.

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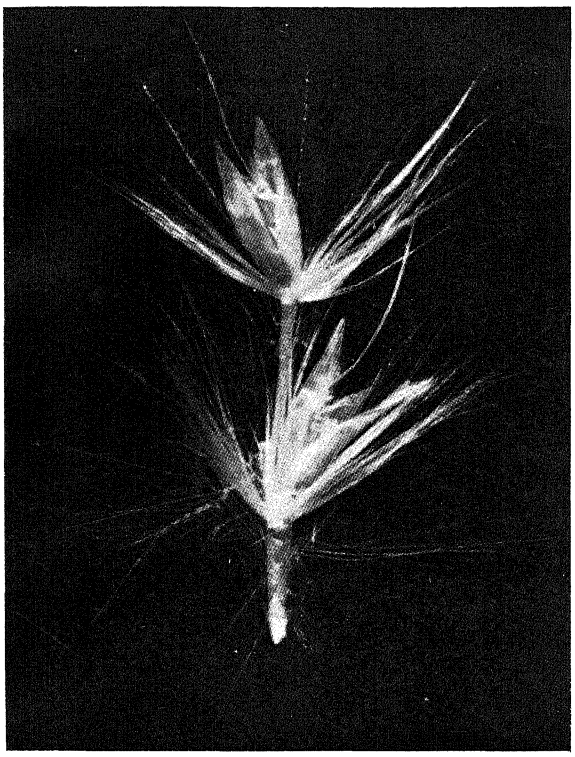

FIG. 1. Spikelets of the intergeneric hybrid S. *spontaneum* (Dehra Dun) × *Sorghum Durra*.

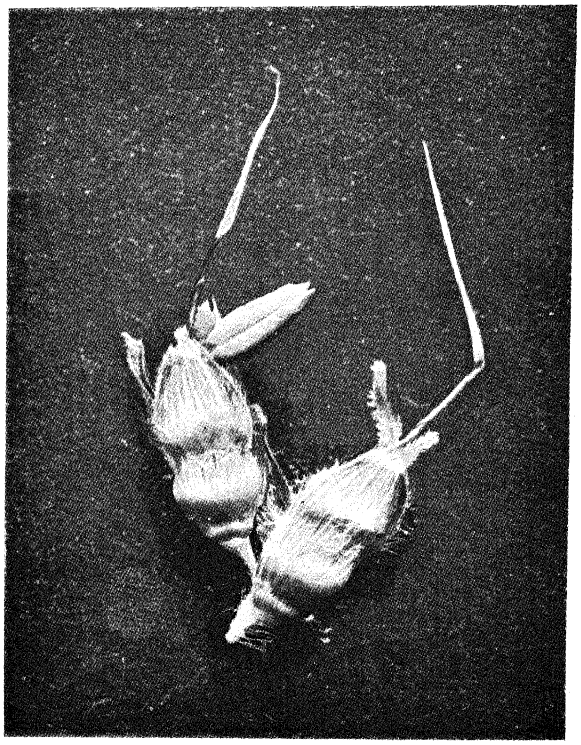

FIG. 2. Spikelets of *Sorghum Durra*.

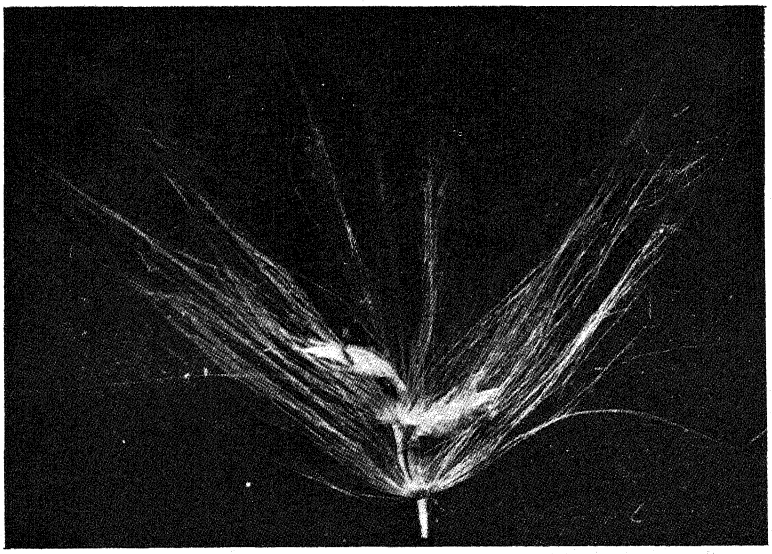

FIG. 3. Spikelets of *S. spontaneum* (Dehra Dun).

STATISTICAL NOTES FOR AGRICULTURAL WORKERS

NO. 19.—THE FREQUENCY DISTRIBUTION OF PLOT YIELDS AND THE OPTIMUM SIZE OF PLOTS IN A UNIFORMITY TRIAL WITH RICE IN ASSAM

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(With three text-figures.)

INTRODUCTION

The tests of significance used in the analysis of the results of agricultural field trials are based on the assumption that yields from individual plots conform to the normal distribution or at least do not deviate so far from normality as to render such tests invalid. Very little is known at present regarding the real nature of the frequency distribution of plot yields of agricultural crops in India. It is desirable therefore to study this question for all important crops under different conditions of cultivation in the different provinces of India. This is the first point considered in the present note.

The effect of the size and shape of plots on the magnitude of the experimental error in field trials also requires careful study. It is possible that there is no single optimum for all purposes. In fact, previous work has shown the wide range of variation under different conditions.

The potato experiment at Ormskirk [1932] showed 195 ft.$\times$8 ft. (=1/28 acre approximately) to be the best plot size, with an experimental error of 4·9 per cent. Another potato experiment (1932) at Saskatchewan showed this optimum size to be 132 ft.$\times$15 ft. 9 in.(=1/21 acre) with an experimental error of 5·1 per cent. In both these experiments, the smallest plot size was found to be the most efficient. Workers on Sorghum in Texas obtained 1/40 to 1/80 acre to be the most suitable. With regard to sugarcane tests the work done at Pusa [Vaidyanathan, 1933] indicated the optimum size to be between 1/20 and 1/30 acre—the ratio of length to breadth being 6·7 to 10·1. Several rice tests have been reported in different parts of India and Ceylon. Lord [1931] working with broadcast rice in Anuradhapura (Ceylon) obtained 1/87 acre to be the most convenient size for practical purposes. Mitra and Ganguli [1934] working

in Assam found 60 ft.×10 ft. (=1/72·6 acre) to be the best for *Aman*, and 60 ft. ×15 ft. (=1/48·4 acre) for *Aus*. In China a recent article by Chien Liang Pan [1935] indicates the optimum size to be 14·2 ft.×3·0 ft. (=1/1025 acre) at Hangchow Central Station, and 14·2 ft.×1·5 ft. (=1/2050 acre) at Wufu.

It is thus clear that the optimum size and shape of plots depends to a very great extent on the crop, the nature of the soil, and the conditions of cultivation. It is, therefore, necessary and desirable to conduct detailed and careful analysis of the results of uniformity trials until sufficient experience has been gathered in this matter. This is the second question studied in this paper.

Material and method

The material for the present study was obtained from an experiment conducted in Karimganj Farm in Assam in 1933. Two 1/10 acre plots measuring 66 ft. × 66 ft. were grown with *As. 2 Kasalath*, a transplanted paddy of *Aus* type. Single seedlings were planted six inches apart. Each field was harvested in the form of a Latin Square with twelve rows and twelve columns with individual plots measuring 5 ft.×5 ft. and containing 100 plants each. Five rows were left out from all sides round the experimental area to eliminate border effects if any; but it was not necessary to eliminate any row round the small plots (5 ft.×5 ft.) as the whole field was uniformly cropped without any special *ails* or spacing anywhere. There were 2×144 or 288 plots in all, and these were harvested, threshed, dried and weighed (in *tolas**) separately.

Frequency distribution of yield data

The data for the two fields were studied separately as well as in combination. The frequency distributions of two sets of 144 observations as well as their combination are shown in Tables I—III.

Table I

Observed and expected frequency distributions of plot-yields of rice (Field I)

Range	Observed (f′)	Expected (f)	$(f-f')^2/f$
Below 39·5	1·5	1·74	0·0331
41·0	6	3·28	2·2556
42·5	5·5	7·24	0·4182
44·0	12	13·13	0·0973
45·5	19	19·68	0·0235
47·0	24	24·34	0·0047
48·5	23	24·85	0·1377
50·0	27·5	20·85	2·1210
51·5	12	14·49	0·4279
53·0	8	8·28	0·0095
54·5	1·5	3·92	1·4940
54·5 and beyond	4	2·20	1·4727
Total	144·0	144·00	$\chi^2=8·4952$

* 1 *tola*=11·6638 grammes=0·4114 ozs.

TABLE II

Observed and expected frequency distributions of plot-yields of rice (Field II)

Range	Observed (f′)	Expected (f)	(f—f′)²/f
Below 38·0	1	1·05	
39·5	0 } 3·5	0·56 } 3·24	0·0209
41·0	2·5	1·63	
42·5	5	3·95	0·2791
44·0	6	8·04	0·5176
45·5	13	13·68	0·0338
47·0	18	19·61	0·1322
48·5	25	23·46	0·1011
50·0	19·5	23·67	0·7346
51·5	22	19·92	0·2172
53·0	20	14·14	2·4285
54·5	6	8·37	0·6711
56·0	5	4·18	0·1609
56·0 and beyond	1	1·74	0·3147
Total	144·0	144·00	$\chi^2 = 5·6117$

TABLE III

Observed and expected frequency distributions of plot-yield of rice (Fields I and II combined)

Range	Observed (f′)	Expected (f)	(f—f′)²/f
Below 38·0	1 } 2·5	1·71 } 3·58	
39·5	1·5	1·87	0·3258
41·0	8·5	5·01	2·4312
42·5	10·5	11·17	0·0402
44·0	18	21·05	0·4419
45·5	32	33·09	0·0359
47·0	42	43·72	0·0677
48·5	48	48·21	0·0009
50·0	47	44·67	0·1215
51·5	34	34·79	0·0179
53·0	28	22·58	1·3010
54·5	7·5	12·33	1·8920
56·0	9	5·64	2·0017
56·0 and beyond	1	2·16	0·6230
Total	288·0	288·00	$\chi^2 = 9·3007$

The frequency constants are shown in Table IV.

TABLE IV

Frequency constants for plot-yields of rice with Standard Errors

	Field I	Field II	Total area
Size of sample . .	144	144	288
Mean . . .	47·1510±0·2827	48·5521±0·2959	47·8515±0·2088
Standard Deviation .	3·3922±0·3998	3·5505±0·4185	3·5424±0·2952
μ_2	11·5085±0·9147	12·6068±1·0021	12·5483±0·9975
μ_3	—1·9838±5·3742	—14·8723±6·1622	—7·2741±6·1203
μ_4	384·2038±62·3655	472·4821±87·5178	446·0285±86·7243
β_1	0·0026±0·0035	0·1104±0·1200	0·0268±0·0247
β_2	2·9009±0·3536	2·9729±0·4639	2·8327±0·2478

It will be seen from Table IV that mean yield in Field II was slightly higher than that of Field I, although the difference was only 1.4011 tolas per plot. The standard deviations of the two fields were also not very different, the ratio of the standard deviations is 1·047, and for $n_1=n_2=143$ is not significant.

The third moment is negligible in Field I, but it is appreciably negative in Field II. The symmetrical form of the distribution in Fig. 1, and the skew curve with a bias on the higher yield side in Fig. 2 show the same results graphically. Fig. 3 shows the frequency distribution of the entire set of results.

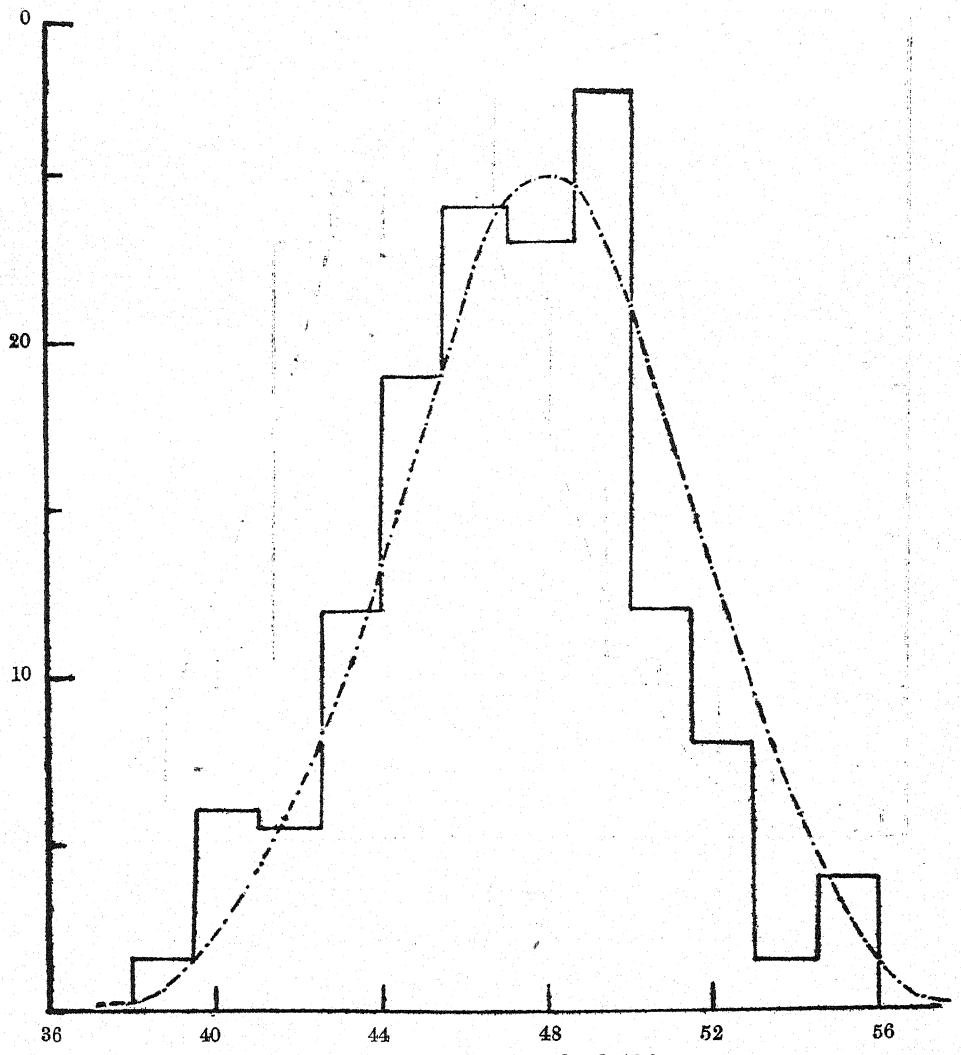

Fig. 1. Frequency distribution of yields of rice in field I.

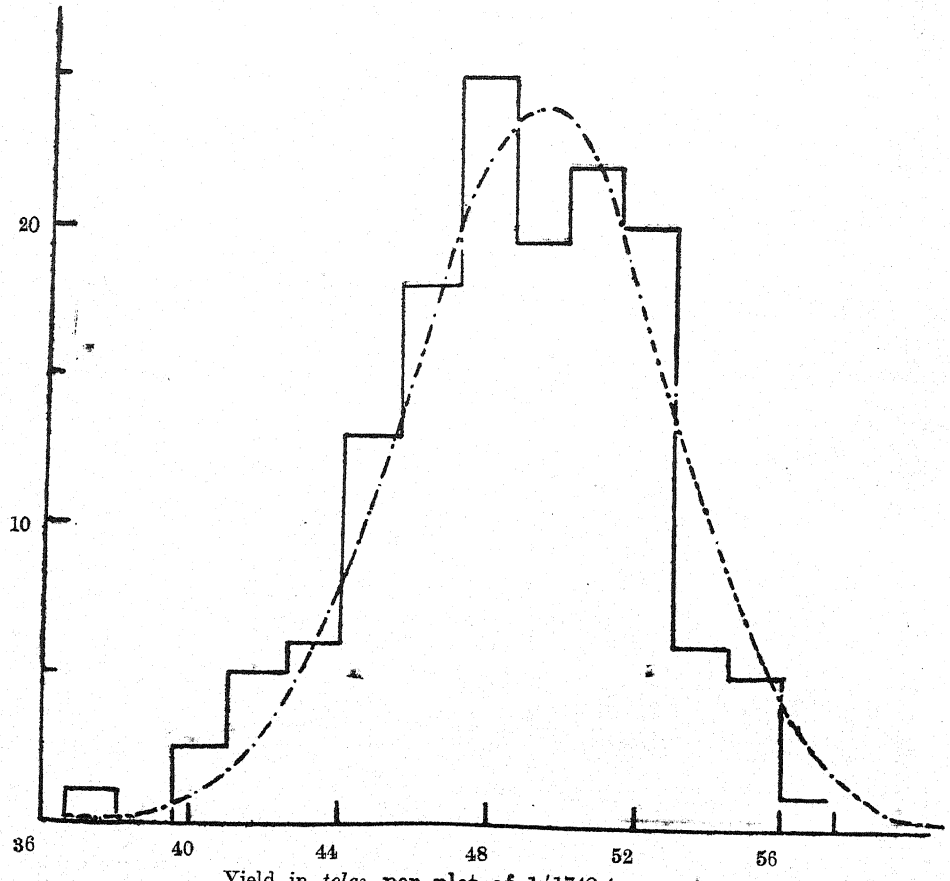

Fig. 2. Frequency distribution of yield of rice in field II.

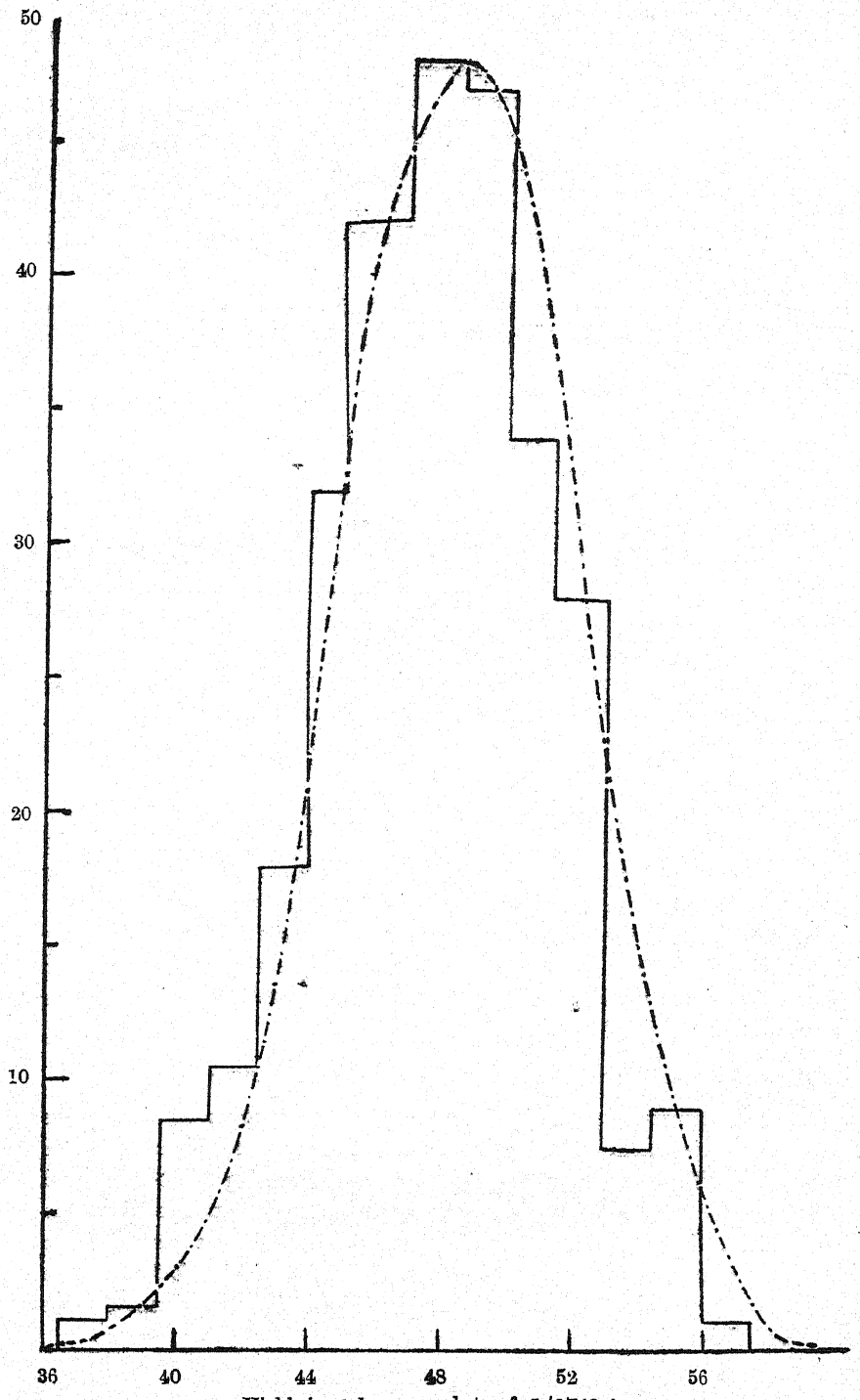

Fig. 3. Frequency distribution of yield of rice in fields I and II.

The standard errors of β_1 and β_2 are large, as the sample is too small to give reliable estimates of third and higher moments. Judged in the light of respective standard errors, the β_1 values are not significantly different from zero, and the β_2 values are also not significantly different from 3, the expected value for the normal curve. The distribution of rice-yields may thus, as far as the fourth moment, be considered to be a sample from a normal population. The observed distributions have been, therefore, graduated by a normal curve, with mean and variance obtained from the sample.

The expected values for each of the frequency distributions (separately, and in combination) have been shown in column (3) and χ^2 values in column (4) in Tables I—III.

TABLE V

Test of goodness of fit by a normal curve

	n	χ^2	P
Field I . . .	9	8·4952	0·48
Field II . . .	9	5·6113	0·78
Total area .	10	9 1307	0·52

The data for testing the goodness of fit are given in Table 5, in which column (2) shows the degrees of freedom on which χ^2 has been calculated, and P (in column 4) gives the corresponding probability of occurrence of systems of deviations as great or greater than the observed system.

We thus find that if our plot yield data are considered to be a sample from a normal population, the chances are 48 in 100 in Field I that χ^2 should be as big as or bigger than the observed value; that is, the fit of a sample from a strictly normal population would be as bad as or worse in forty-eight per cent of cases. Hence, the hypothesis that the yield data conform to a normal distribution may be accepted as true for all practical purposes. In the same way the assumption of a normal distribution for the uniformity trial data is statistically justified for field II, as well as for the combined experiment.

Standard Deviation of plots of different shape and size

The lay-out of the experiment was in the form of two Latin Squares with twelve rows and twelve columns each. It was thus possible to conduct the analysis for plots of twenty-five different sizes and shapes, the smallest being 5 ft. × 5 ft. and the largest 30 ft. × 30 ft. In each case, the effect of soil heterogeneity was eliminated by subtracting the sums of squares between rows and columns within each Latin Square from the total sum of squares, and the variation between the two Latin Squares was also allowed for. The analysis of variance for the 5 ft. × 5 ft. plot is shown in Table VI.

TABLE VI

Analysis of variance of rice yield

(Plot-size 5 ft. × 5 ft.)

	D F.	Sum of squares	Mean Square	Ratio of variance
Field	1	131·0176	131·0176	14·24
Row	22	658·7119	29·9414	3·25
Column	22	566·3368	25·7425	2·80
Soil-difference	45	1356·0663	30·1348	3·27
Residual	242	2227·1656	9·2031	..
Total	287	3583·2319	12·4851	

The mean yield of plot of this size (5 ft. × 5 ft.) is 47·85, so that the residual standard deviation (3·03) is 6·33 per cent of mean. Without eliminating the heterogeneity, the standard deviation is 3·54, or 7·37 per cent of mean. The standard deviation before and after elimination of the soil differences are shown as percentages of mean for various plot sizes in Tables VII (*a*) and (*b*).

TABLE VII

Standard deviation of a single plot as per cent of the mean

	Length of plot				
	5 ft.	10 ft.	15 ft.	20 ft.	30 ft.
(*a*) *Before eliminating fluctuations due to fields, rows and columns*					
Width of plot—					
5 ft.	7·37	5·70	4·92	4·50	3·94
10 ft.	5·97	5·04	4·33	4·04	3·36
15 ft.	5·48	4·57	4·03	3·54	3·27
20 ft.	4·87	4·15	3·38	3·24	3·05
30 ft.	4·16	3·53	3·07	2·70	2·94
(*b*) *After eliminating fluctuations due to fields, rows and columns*					
5 ft.	6·33	4·85	4·14	3·89	3·20
10 ft.	4·98	4·18	3·58	3·57	2·14
15 ft.	5·21	4·56	4·40	4·16	4·15
20 ft.	3·62	2·96	1·83	2·18	1·50
30 ft.	2·76	2·18	1·74	1·49	1·65

SYSTEMATIC SOIL-DIFFERENCES

As plot size increases, either the row or the column or both increase in size, and the amount of systematic fertility differences eliminated is different in different cases. The total amount of variation eliminated is shown in Table VIII, in which column (1) gives the size of plot, column (2) the average sum of squares for variation due to field, rows and columns, and column (3) the mean residual variance.

TABLE VIII

Mean Squares of soil-heterogeneity and residual error for different plot sizes

Plot size	Mean Square			
	Soil-heterogeneity		Residual	
5 ft. × 5 ft.	**30·1348	(45)	9·2031	(242)
5 ft. × 10 ft.	**72·9528	(33)	22·7157	(110)
10 ft. × 5 ft.	**66·3345	(33)	21·5476	(110)
15 ft. × 5 ft.	**95·2434	(29)	35·4134	(66)
5 ft. × 15 ft.	86·6525	(29)	56·02	(66)
10 ft. × 10 ft.	**183·0614	(21)	64·07	(50)
20 ft. × 5 ft.	*121·1467	(27)	55·5440	(44)
5 ft. × 20 ft.	**166·9010	(27)	47·9760	(44)
15 ft. × 10 ft.	*277·8899	(17)	105·7872	(30)
10 ft. × 15 ft.	209·9972	(17)	171·58	(30)
30 ft. × 5 ft.	*192·8350	(25)	84·3067	(22)
5 ft. × 30 ft.	**240·4479	(25)	63·0663	(22)
20 ft. × 10 ft.	365·1513	(15)	187·25	(20)
10 ft. × 20 ft.	**471·4306	(15)	128·4384	(20)
15 ft. × 15 ft.	291·6506	(13)	358·8998	(18)
10 ft. × 30 ft.	*700·9708	(13)	151·742	(10)
30 ft. × 10 ft.	*631·9015	(13)	156·59	(10)
20 ft. × 15 ft.	347·4507	(11)	571·4876	(12)
15 ft. × 20 ft.	**774·6905	(11)	110·2575	(12)
20 ft. × 20 ft.	*1079·1531	(9)	278·6736	(8)
30 ft. × 15 ft.	630·3538	(9)	1279·3644	(6)
15 ft. × 30 ft.	*1193·2188	(9)	224·1087	(6)
30 ft. × 20 ft.	*2032·3196	(7)	295·9856	(4)
20 ft. × 30 ft.	1654·4775	(7)	294·8038	(4)
30 ft. × 30 ft.	*5619·4918	(3)	808·1761	(4)

In column 1, 1st figure represents lengths in feet north to south, 2nd figure represents widths in feet east to west.

Double stars (**) indicate significance at one per cent and single star (*) at five per cent.

The figures in brackets in columns (2) and (3) give the degrees of freedom on which the variances have been calculated. Fisher's z-test shows that in practically all cases elimination of differences due to soil-heterogeneity lead to an appreciable improvement in accuracy.

DISCUSSION OF RESULTS

The first point to be noticed in Table VII (*a*) is that the first four figures in all the five columns show a steady decrease, the error for one-row wide plots being always the highest in each column. Compared to errors for one-row wide plots in Table VII (*b*) errors for two-row wide plots are much lower. The errors are again high for three-row plots, but decreased for four-row plots. This effect was noticed by Justesen [1932] and Kalamkar [1932] for potato, and was

attributed to the influence of competition between rows. Justesen suggested [1932, p. 369] that " a high yielding row will probably depress the yields of the adjacent row, in this way giving a high variation between rows. When these rows are paired, the pairs will tend to give an average with less variation than single row." It is possible that such a competitive effect also works between successive series of rice plots, but while the figures in the alternate rows of Table VII show some evidence of such an effect, yields in alternate columns are apparently not affected by competition, although conditions are presumably same along both ways for square-sized plots regarding spacing of plants.

The smallest error (1·50 per cent after elimination of soil-differences) occurs in six-row long and four-row wide as well as in four-row long and six-row wide plots, the size in both cases being 30 ft.×20 ft. (=1/72·6 acre). The bigger plots as a rule show smaller errors than smaller ones, but the present experiment does not fully corroborate the claims made by a number of field workers that longer plots invariably show lower residual errors.

Efficiency of different plot sizes

In discussing the relative efficiency of plot-size, it is necessary to remember that, given a definite total area, the number of replications is reduced as the plot size is increased. Consider a fixed total size of field A. If the plot size is doubled the number of replications will be halved at the same time. If there is gain in precision due to increase in the size of the plot, there will be a loss of accuracy due to the consequential reduction in the number of replications. In determining the optimum size of plots, we must, therefore, take into consideration both the factors :—

(1) The increase of precision due to increased size of individual plots.

(2) The loss of precision due to the reduction in the number of replications.

Let e_a be the percentage error of a plot area " a ," and n_a the available number of replications, then the standard error for the given plot-size " a " may be written —

$$E_a = \frac{e_a}{\sqrt{n_a}}$$

But $a . n_a$ = total area of field=A= constant. Thus

$$n_a = A/a \text{ or } 1/\sqrt{n_a} = \sqrt{(a/A)}$$

so that $E_a = e_a \sqrt{(a/A)}$.

Now E_a takes into consideration both sets of factors, errors appropriate to the given plot-size as well as to the available number of replications, and is thus a suitable criterion for testing the net efficiency of different plot sizes, the one with the smallest value of E_a being the most efficient. The values of E_a (where " a " is measured in multiples of the smallest plot as unit) are shown in Table IX (a) and (b).

TABLE IX

Standard deviation of a single plot after correcting for its size in per cent of the mean

		Length of plot				
		5 ft.	10 ft.	15 ft.	20 ft.	30 ft.
		(*a*) *Before eliminating fluctuations due to fields, rows and columns*				
(Width of plot)	5 ft.	7·37	8·06	8·52	9·00	9·65
	10 ft.	8·44	10·08	10·60	11·42	11·64
	15 ft.	9·49	11·19	12·09	12·26	13·87
	20 ft.	9·74	11·74	11·71	12·96	14·94
	30 ft.	10·19	12·23	13·02	13·23	17·64
		(*b*) *After eliminating fluctuations due to fields, rows, and columns*				
(Width of plot)	5 ft.	6·33	6·86	7·17	7·78	7·84
	10 ft.	7·04	8·36	8·77	10·10	7·41
	15 ft.	9·02	11·17	13·20	14·41	17·60
	20 ft.	7·24	8·37	6·34	8·72	7·35
	30 ft.	6·76	7·55	7·38	7·30	9·90

Table IX differs considerably from Table VII. Plots of size 30 ft.×20 ft. (six-row×four-row and four-row×six-row) showed the smallest residual standard deviations in Table VII, but in Table IX the highest precision is given by the smallest plot 5 ft.×5 ft. The smallest plot-size in this experiment provided such a large number of replications that the gain in accuracy due to this factor more than compensated for the loss of precision due to the reduction in the actual size of plots.

The results given in Table IX are shown in percentage form in Table X in which the residual errors for the different plot sizes are shown expressed as percentage of the residual error for the smallest plot. (That is, the residual error of the unit plot (5 ft.×5 ft.) after eliminating soil heterogeneity is taken=100).

TABLE X

Percentage standard deviation of a single plot expressed as a percentage of the minimum plot (5 ft.×5 ft.) with the errors for fields, rows and columns eliminated

		Length of plot				
		5 ft.	10 ft.	15 ft.	20 ft.	30 ft.
	(a) Without eliminating the fluctuations due to fields, rows and columns					
(Width of plot)	5 ft.	116·42	127·33	134·60	142·18	152·45
	10 ft.	133·33	159·24	167·46	180·41	183·89
	15 ft.	149·92	176·78	191·00	193·68	219·12
	20 ft.	153·87	185·47	184·99	204·74	236·02
	30 ft.	160·98	193·21	205·69	209·01	278·68
	(b) Eliminating the fluctuation due to fields, rows and columns					
(Width of plot)	5 ft.	100·00	108·37	113·27	122·91	123·86
	10 ft.	111·22	132·07	138·55	159·56	117·06
	15 ft.	142·50	176·46	208·53	227·65	278·04
	20 ft.	114·38	132·23	100·16	137·76	116·12
	30 ft.	106·79	119·27	116·59	115·33	156·40

Fisher presents similar results in terms of "efficiency", where the "efficiency" Fa is defined to be inversely proportional to E_a^2, that is

$$Fa \propto 1/E_a^2$$

Thus in order to express the results in terms of Fa, we have simply to take the square of the reciprocals of the figures given in Table X. It is clear that the unit plot (5 ft.×5 ft.) would give the highest value of Fa, showing its maximum efficiency in the Fisherian sense.

For the convenience of comparison, the values of Fa are given in Table XI.

TABLE XI

Relative efficiency of plots of different shapes and sizes (Fa).

		Length of plot				
		5 ft.	10 ft.	15 ft.	20 ft.	30 ft.
	(a) Without eliminating the fluctuations due to fields, rows and columns					
(Width of plot)	5 ft.	73·78	61·67	55·19	49·47	43·02
	10 ft.	56·25	39·42	35·66	30·72	29·57
	15 ft.	44·48	32·00	27·40	26·65	20·83
	20 ft.	42·23	29·07	29·21	23·85	17·95
	30 ft.	38·58	26·79	23·63	22·89	12·87

TABLE XI—*contd.*

Relative efficiency of plots of different shapes and sizes (Fa).

(Width of plot)	Length of plot: 5 ft.	10 ft.	15 ft.	20 ft.	30 ft.
(b) After eliminating fluctuations due to fields, rows and columns					
5 ft.	100·00	85·15	77·95	66·18	65·18
10 ft.	80·84	57·33	52·08	39·27	72·97
15 ft.	49·23	32·11	22·99	19·28	12·93
20 ft.	76·44	57·13	99·68	52·80	79·15
30 ft.	87·68	70·29	73·56	75·18	40·88

It is worth noting that the results for net efficiency (as given in Table X or Table XI) show considerable irregularities, although a few systematic features are also present. Thus one-row wide plots are usually considerably more efficient than two-row and three-row wide plots ; also one-column long plots are on the whole the most efficient. The three most efficient plot sizes on the other hand are quite irregular and are given by one-column × one-row (5 ft. × 5 ft. = 1/1742·4 acre with a value of Ea=100), three-column × four-row (15 ft. × 20 ft. = 1/145·2 acre ; Ea=100·2), and one-column × six-row (5 ft. × 30 ft. = 1/290·4 acre ; Ea=106·8).

It is scarcely necessary to add that convenience of agricultural operations such as the laying out of the field, tillage, ploughing, harvesting, etc., as well as the relative economic cost of such operations must also be taken into consideration in fixing the optimum size of plots. The results of the present experiment show however that fortunately the range of choice from the point of view of purely statistical efficiency is likely to be quite wide. For example in this particular experiment, the difference in statistical efficiency between plot-sizes of 5 ft. × 5 ft. (=1/1742·4 acre), 5 ft. × 30 ft. (=1/290·4 acre), and 15 ft. × 20 ft. (=1/145·2 acre) is quite small, so that the final choice can be easily made from the point of view of practical convenience.

SUMMARY

The present paper gives an analysis of a uniformity trial on transplanted rice (*As.2 Kasalath* of *Aus* type) conducted in the Karimganj Farm, Assam, in 1933-34 in the form of two Latin Squares with 12 rows × 12 columns each.

(1) The observed frequency distributions of the yield figures for each Latin Square as well as for both combined are not significantly different from normal, and can be graduated quite satisfactorily by the normal curve. The assumption of a normal distribution of plot yields is, therefore, justified within the limits of sampling errors of the present experiment.

(2) The results were analysed in detail by the method of analysis of variance for all possible (25 different) size and shape of plots. In practically every case the

elimination of variation due to systematic differences in soil-fertility led to an appreciable decrease in the residual errors.

(3) There was some evidence of the presence of competition between rows, but not between columns, although conditions of spacings between plants were apparently the same along both directions so far as the lay-out was concerned.

(4) The smallest residual variance occurred with plots of size 30 ft.×20 ft. (6-row×4-row=1/72·6 acre) which was the second largest size analysed in this experiment.

(5) Taking into consideration the effect of reduction in the number of replications due to an increase in the size of individual plots, the net residual error is lowest in the case of the smallest plot-size (5 ft.×5 ft. =1/1742·4 acre). The difference in net efficiency of comparison is, however, small with plot sizes of 5 ft.× 5 ft, 5 ft.×30 ft., and 15 ft.×20 ft., so that the range of choice is fairly wide for fixing the optimum size of plots from considerations of practical convenience.

Fairly small-sized plots (of the order of 1/1742·4 or 1/145·2 acre) are thus statistically quite efficient for experiments on rice.

It is a pleasure to express our indebtedness to Dr. S. K. Mitra, Economic Botanist, Assam, for his kind encouragement in the present investigation. Our thanks are also due to Mr. S. K. Banerjee of the Statistical Laboratory for his valuable help in the statistical analyses.

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STATISTICAL NOTES FOR AGRICULTURAL WORKERS

No. 20—STUDIES IN TILLER VARIATION

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(With three text-figures)

INTRODUCTION

Rice, like many cereals, has the capacity of producing side-shoots ; this is called tillering in rice. The shoots are produced from the axil of the leaf, the tillering zone being confined to a small portion of the base of the rice plant where internodes are close, although in deep water paddies the tillers come out from any node. Usually the tillers are produced on alternate sides of the main stem ; these secondary shoots in turn produce tertiary ones and so on.

Under normal conditions of cultivation, rice plants begin to tiller a week after transplanting. In early *Aus* varieties tiller formation may cease from the fifth or the sixth week but in late varieties it may extend up to the eighth or the ninth week. This limiting period may be termed the critical stage of tiller formation, after which the tillers grow up and produce stems each carrying an ear.

The average number of tillers per plant is a varietal characteristic, different varieties producing different number of tillers. In fact, the yield of a variety depends a good deal on its tillering capacity, which is, therefore, considered to be an important economic factor in the study of the rice plant. All tillers, however, are not equally effective in contributing to the final yield ; thus, under heavy manuring or very wide spacing, tillering may continue till the flowering stage, but these tillers do not ripen with the earlier tillers and may not yield grains. They mostly waste the energy of the plant without any corresponding return.

In classifying rice considerable weight is given to the study of the nature and number of tillers formed, and recent work on rice in Assam, Bengal and Madras go to show the importance of tiller studies. In the present investigation, range of

variation of tillers from plant to plant of a given variety of rice, the nature of the distribution of tillers, and the effect of soil heterogeneity on tiller numbers have been studied from a statistical standpoint, and an attempt has been made to determine the order of accuracy of the estimates for a given number of tiller counts.

The present tiller test was conducted at the Government Agricultural Farm, Bankura, in 1933-34. Three blocks of land measuring 1/10 acre each were transplanted with single plants of *Jhingasail* a local variety of rice grown very frequently in Western Bengal. The blocks were laid out in the form of 5×5 Latin Squares, and each plot contained ninety plants in nine rows of ten columns each with a spacing of nine inches from plant to plant. The dates of transplanting were different in the three blocks and are given below :—

Field No. 37/B	13th July, 1933.
Field No. 36/B	27th July, 1933.
Field No. 35/B	7th August1933.

The total number of plants in each block was $90 \times 25 = 2,250$, so that in three blocks there were 6,750 plants altogether. The number of tillers grown on each of these plants was counted by one of us (K. C. B.) with the help of Mr. J. R. Pal, Assistant Rice-Research Officer, Bankura, and these records form the material for the present note.

Range of variation

Omitting three plants in blocks 37/B and 35/B, and two plants in block 36/B which had died, tiller formation ranged up to seventeen per plant in 37/B, and fourteen in each of 36/B and 35/B. The range of mean tillers per plot of ninety plants in the three blocks together with the range of standard deviations are shown in Table I

Table I

Range of variation of mean and S. D. of tillers

Block	Range of variation	
	Mean	S. D.
37/B	5·24—9·33	1·10—2·17
36/B	5·42—8·41	1·02—2·08
35/B	3·87—7·60	1·11—2·03

The mean tiller numbers per plant for each of the twenty-five plots in blocks 37/B, 36/B and 35/B together with the standard deviations are shown in Appendix I. The statistical analysis is given in Table II.

TABLE II

Analysis of variance

	D. F.	Sum of squares	Mean square	Ratio
		(*a*) *Field 37/B*		
Rows	4	1323·97	330·99	5·42
Columns	4	156·61	39·15	0·64
Residual	16	976·97	61·06	
Total	24	2457·55		
		(*b*) *Field 36/B*		
Rows	4	91·90	22·98	0·71
Columns	4	244·50	61·13	1·90
Residual	16	514·59	32·16	
Total	24	850·99		
		(*c*) *Field 35-B*		
Rows	4	1067·33	266·83	6·54
Columns	4	153·58	38·40	0·94
Residual	16	653·05	40·81	
Total	24	1873·96		
		(*d*) *Three fields combined*		
Rows	12	2483·20	206·93	4·63
Columns	12	554·69	46·22	1·03
Field	2	24924·12	12462·06	278·92
Residual	48	2144·61	44·68	
Total	74	30106·62		

It will be seen from the above tables that the mean square deviation " between columns " were always of the same order as the residual variance. The row variance, however, was very large in blocks 37/B and 35/B, but not in 36/B. The variances for tillers per plot were roughly of the same order in all three fields, and were 61·06, 32·16 and 40·81 respectively ; pooling these results, the variance was 44·68, so that the standard deviation between tillers per plant in the plots of ninety plants each is 6·7 approximately.

Since data for individual plants have been recorded, it is possible to make an estimate of systematic soil differences within the same plot. There are ninety plants in each plot arranged in ten rows and nine columns ; the mean variances of tillers per plant between rows and columns are shown in Appendix II (*a*)-(*c*) together with the residual and total variances. It will be seen that, with the exception of a few plots, the row and column variances were not significant. Hence as

far as the tiller counts are concerned each plot appeared fairly homogeneous within itself, although the whole field of twenty-five plots showed appreciable soil differences between rows (Table II).

The frequency constants together with the standard errors are shown in Table III. We notice that block 37/B gives the highest mean tiller per plant, block 36/B comes next, with block 35/B as significantly the lowest. The standard deviations in the three blocks, though statistically differentiated, are fairly close to each other and the pooled standard deviation of tillers per plant is 1·92.

TABLE III

	Frequency constants with standard errors			
	Observed values			Expected
	37/B	36/B	35/B	
Size of sample	2250	2250	2250	
Mean	7·2249 ±0·0425	6·6498±0·0380	5·9334±0·0409	
Standard Deviation	2·0160±0·0301	1·8013±0·0269	1·9402±0·0289	
β_1	0·0551±0·0263	0·0547±0·0202	0·2637±0·0576	0·0000
β_2	3·3436±0·2003	3·0497±0·1259	3·2809±0.1841	3·0000

The β/values in the first two blocks (37/B and 36/B) are not significantly different from zero so that these distributions may be taken to be symmetrical. The distribution of tillers in block 35/B is, however, appreciably skew, the bias being on the side of lower value. The β_2 values in the three cases are not significantly different from 3 (=the value of β_2 in the case of normal curves). The histograms for these three observed distributions are shown in Figs. 1—3.

So far as the first four moments are concerned, the distribution of the number of tillers per plant in the first two blocks cannot be distinguished from a normal type. The distribution in the third block is slightly skew and rather abruptly rises to a maximum and then slowly tails off at either end; but even then, the deviations of β_1 and β_2 from Gaussian values are not very marked.

The observed frequencies were next graduated by a normal curve with mean value and variance based on the sample. The observed and expected frequencies are given in Table IV and also in Figs. 1—3.

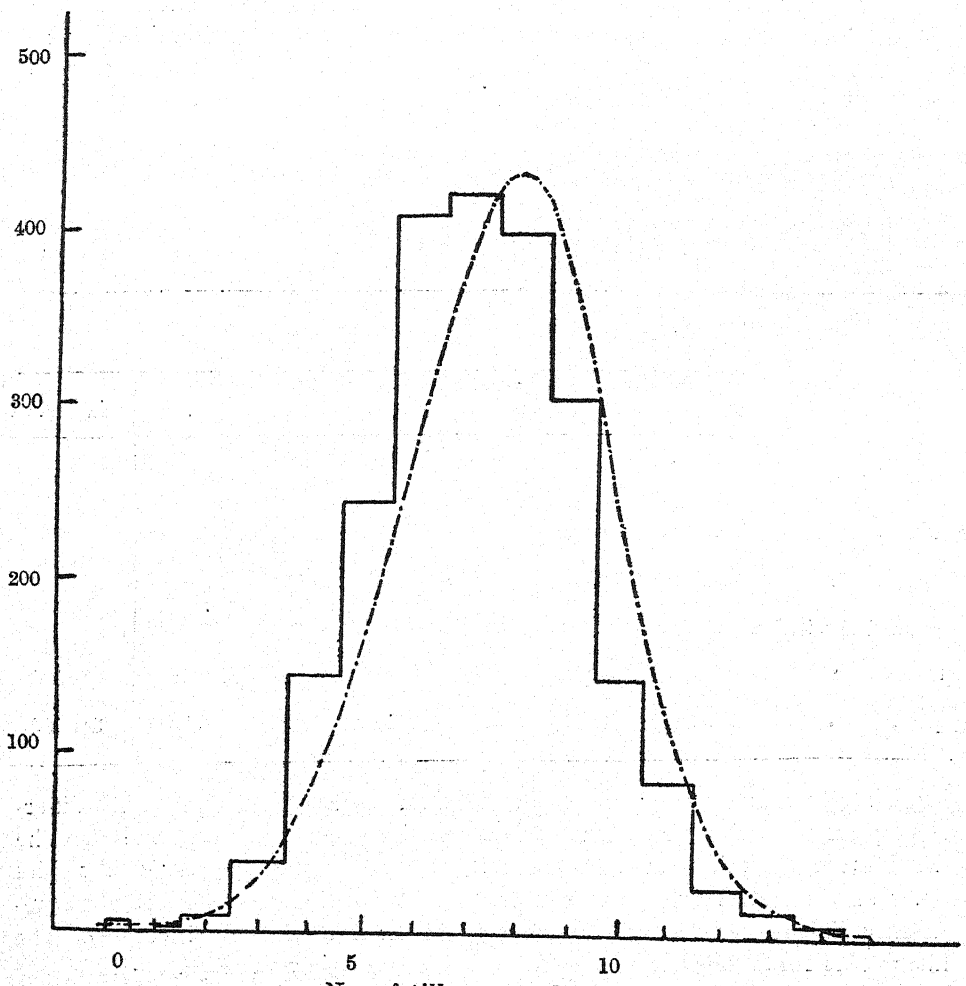

Fig. 1. Frequency distribution of tillers in field 37/B.

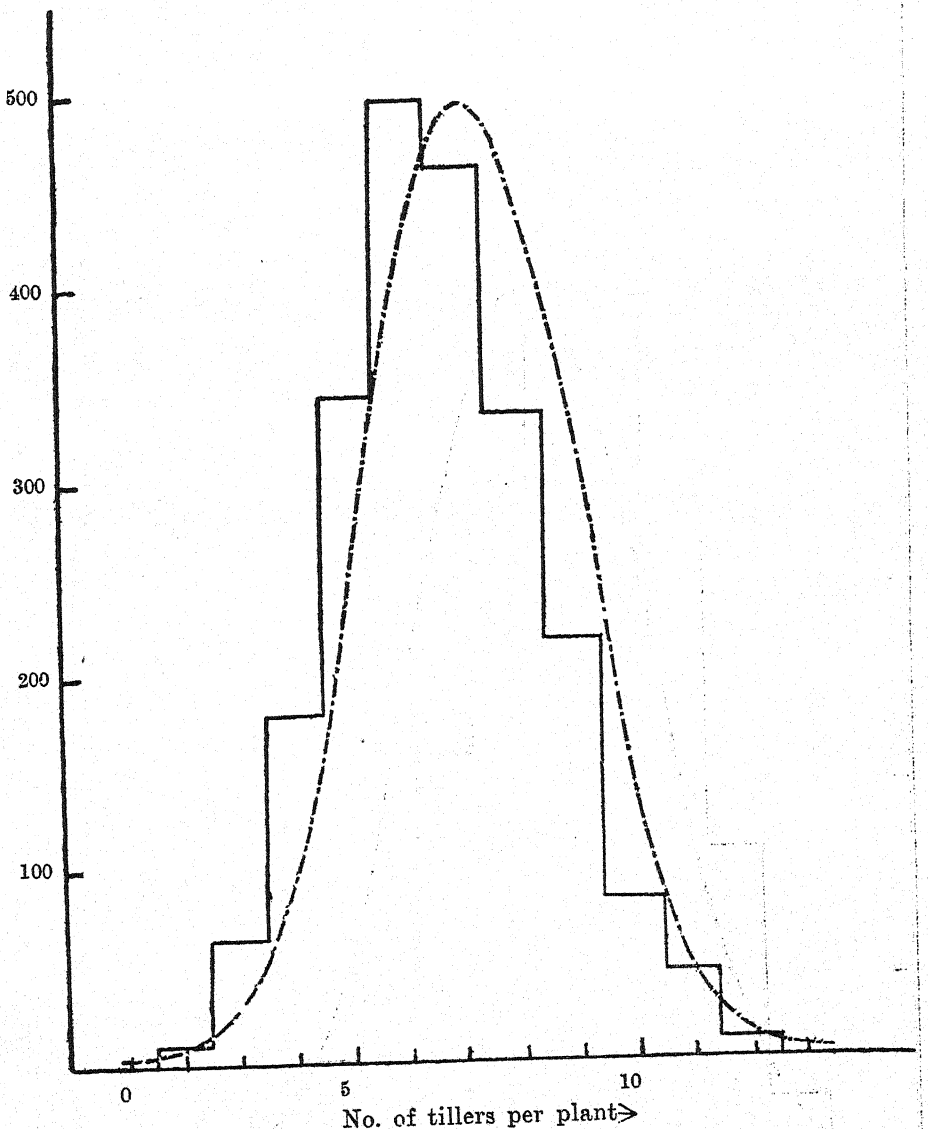

Fig. 2. Frequency distribution of tillers in field No. 38/B.

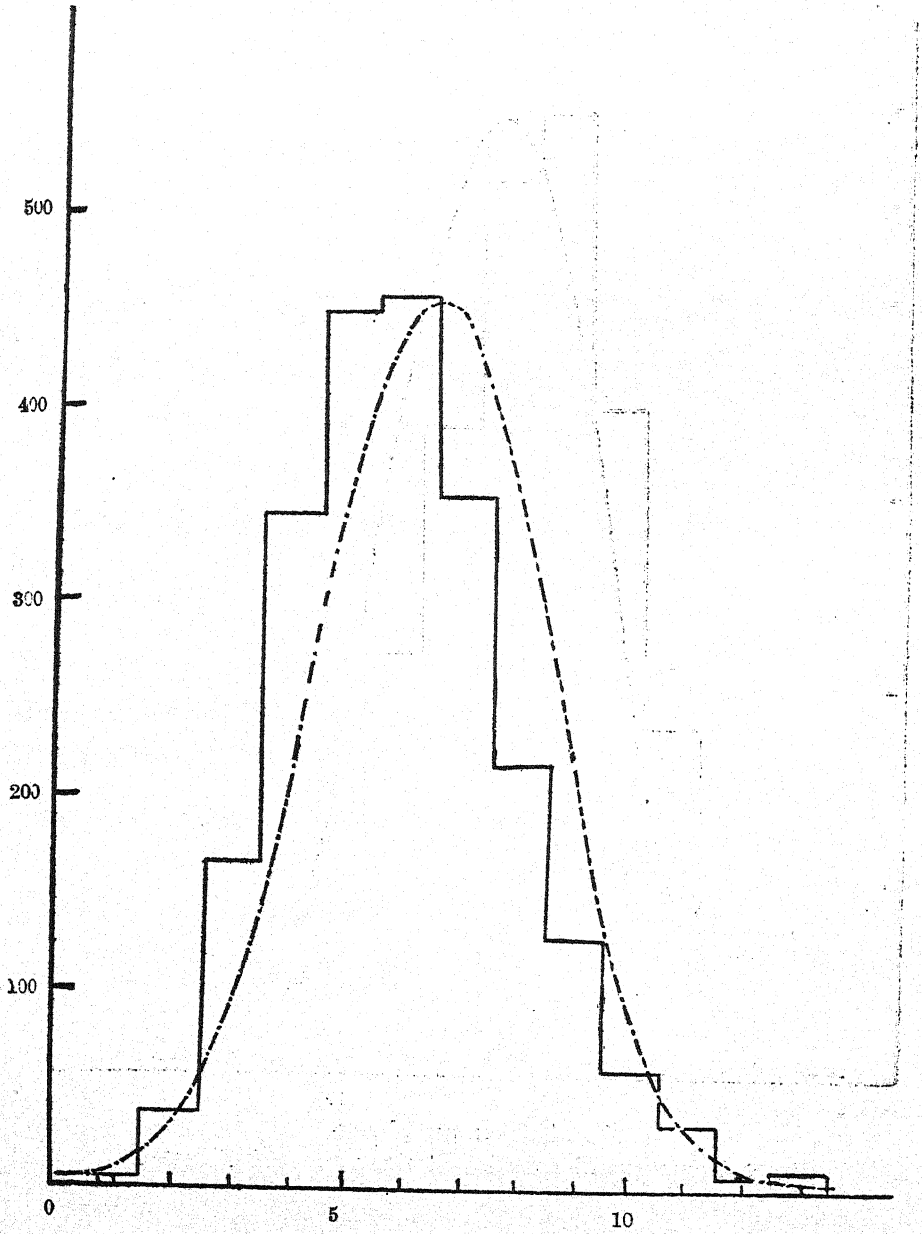

Fig. 3 Frequency distribution of tillers in field No. 35/B.

TABLE IV

Observed and calculated frequency distribution of tillers and (P,χ^2) test

No. of tillers	Field 37/B			Field 36/B			Field 35/B		
	Observed (f′)	Calculated (f)	$(f-f')^2/f$	Observed (f′)	Calculated (f)	$(f-f')^2/f$	Observed (f′)	Calculated (f)	$(f-f')^2/f$
0	3	0·45	14·45	2	0·22		0	2·70	2·70
1	1	1·80	0·36	0 } 11	1·80 } 11·02	0·02	3	9·90	4·81
2	7	8·55	0·28	9	9·00		38	35·55	0·17
3	37	29·70	1·79	63	36·90	18·46	166	99·00	45·34
4	144	82·80	45·23	180	110·93	43·01	342	212·18	79·43
5	245	180·00	23·47	344	245·70	39·33	447	351·00	26·26
6	410	307·80	33·93	498	403·20	22·29	455	442·80	0·34
7	421	413·58	0·13	463	490·27	1·52	353	441·23	17·64
8	400	436·72	3·09	336	442·13	25·48	215	332·78	41·69
9	305	362·47	9·11	219	293·85	19·07	127	194·40	23·37
10	145	236·25	35·24	82	145·35	27·61	59	87·97	9·54
11	87	121·05	9·58	44	52·87	1·49	33	30·37	0·23
12	26	48·60	10·51	8	14·40		4	7·87	1·90
13	12	15·52	0·80	1 } 10	2·92 } 17·78	3·40	7	1·80	15·02
14	5	3·82	0·36	1	0·46		1	0·23	2·58
15	1	0·67	0·16						
16	0 } 1	0·22	2·76						
17	1								
	2250	2250·0	$\chi^2=191·26$ $P<10^{-6}$	2250	2250·00	$\chi=201·68$ $P<10^{-6}$	2250	2249·78	$\chi^2=271·02$ $P<10^{-6}$

A glance at the diagrams shows that the fit is totally inadequate. The same conclusion is reached from the very high values of (P, χ^2). In fact, the odds against the samples having been drawn from a normal population are considerably greater than a million to one.

At first sight it may appear a little strange that an observed distribution for which the two conditions $\beta_1=0$, and $\beta_2=3$ are approximately satisfied does not admit of even a moderately adequate fit by a normal curve. The reason, however, is clear. If an observed distribution is truly normal not only its first four moments but the higher moments also must agree with the theoretical values within the limits of errors of sampling. The method of classifying an observed distribution with the help of moment functions is only tentative; actual graduation by the expected type and comparison of observed values by the (P, χ^2) test is always desirable.

In the present case the χ^2-test shows conclusively that the distribution of tillers per palnt cannot possibly be considered to be of the normal type. It remains now to be examined how far the use of Fisher's z and t-tests are adequate when the population to be sampled is of this type.

Summary

The number of tillers in each of 6,750 rice plants grown in Bankura Farm in 1934 were counted and the observations have been analysed for investigating into (*i*) the range of mean and standard deviation of tillers from plot to plot, (*ii*) the nature of frequency distribution of tillers and (*iii*) the effect of soil differences on tiller number.

(1) Mean tillers per plant based on a sample of 90 ranged between 3·87 and 9·33.

(2) Standard deviation of tiller number varies between 1·02 and 2·17. The pooled standard deviation is 1·92 tillers per plant.

(3) The variance between rows was significant. The tiller numbers within plots of 1/250 acre did not show appreciable heterogeneity.

(4) The distribution of tillers was not significantly different from the normal type as far as the first four moments. The higher moments were, however, significantly different from those of normal distribution, and thus the normal curve was inadequate for graduating the frequency distribution of tillers. This was also confirmed by (P, χ^2) values.

Acknowledgments

We have great pleasure in acknowledging our gratefulness to Mr. K. McLean, Director of Agriculture, Bengal, and to Mr. S. G. Sarangapani, Economic Botanist, Bengal, for their kind interest in this investigation. Thanks are also due to Mr. J. R. Pal, B.A., Assistant Rice Research Officer, Bankura and to Messrs.

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Appendix I

Mean value of number of tillers based on 90 plants and their standard deviations

Serial No.	Field No. 37/B		Field No. 36/B		Field No. 35/B	
	Mean	S. D.	Mean	S. D.	Mean	S. D.
1	6·61	1·488	5·57	1·955	4·96	1·105
2	5·24	1·621	6·64	1·668	3·87	1·230
3	5·93	1·344	7·06	1·702	4·16	1·307
4	6·44	1·175	6·74	1·582	4·91	1·547
5	6·53	1·833	8·41	1·885	4·87	1·127
6	5·41	1·994	7·38	1·901	5·62	1·352
7	6·09	1·465	6·44	1·661	6·40	1·890
8	7·07	1·537	6·58	1·795	6·53	1·157
9	7·33	1·542	6·56	1·017	6·64	1·728
10	7·54	1·627	5·50	1·753	3·98	1·549
11	6·29	2·088	6·51	1·327	5·71	1·797
12	7·27	1·724	5·70	1·696	6·68	1·858
13	7·37	1·487	6·02	1·422	4·62	1·409
14	7·49	1·734	6·63	1·622	7·60	1·756
15	6·52	1·878	7·86	1·716	6·56	1·472
16	8·60	1·872	6·56	1·480	5·83	1·655
17	6·69	2·174	7·36	1·448	5·69	1·658
18	9·33	2·033	5·42	1·591	6·58	1·542
19	8·44	1·826	6·14	1·596	7·20	1·477
20	7·60	2·077	6·22	1·364	6·77	1·897
21	8·64	1·815	6·78	1·638	7·44	2·026
22	9·13	1·691	6·61	2·080	6·74	1·777
23	7·72	2·129	7·56	1·586	6·70	2·003
24	7·74	1·102	7·32	1·787	6·77	1·726
25	7·24	1·515	6·60	2·010	5·53	1·634

Appendix II

Mean variance of rows, columns and residue

Serial No. of the plot.	Row (9)	Column (8) .	Residue (72)	Total (89)
		(*a*) *Field 37/B*		
1	2·48	2·59	2·16	2·2403
2	2·77	3·65	2·55	2·6587
3	2·94	1·73	5·05	1·8270
4	*2·19	**3·54	1·04	1·3958

** Indicate significance at one per cent.
* Indicates significance at five per cent.

APPENDIX II—*contd.*

Serial No. of the plot	Row (9)	Column (8)	Error (72)	Total (89)
		(*a*) *Field No. 37/B*—contd		
5	5·77	4·73	2·97	3·3978
6	2·08	2·45	4·43	4·0201
7	3·11	1·68	2·13	2·1718
8	2·45	4·09	2·17	2·3888
9	2·78	3·80	2·20	2·4045
10	3·68	1·80	2·67	2·6778
11	3·89	2·57	4·70	4·4100
12	2·84	6·13	2·62	3·0067
13	1·03	2·91	2·29	2·2348
14	3·22	2·13	3·27	3·0388
15	2·89	4·26	3·56	3·5782
16	2·03	6·53	3·34	3·5461
17	3·21	**12·77	3·97	4·7785
18	4·36	2·42	4·39	4·1798
19	3·17	3·56	3·37	3·3733
20	4·52	1·28	4·67	4·3620
21	3·01	3·48	3·35	3·3328
22	2·20	2·61	3·00	2·8921
23	4·94	7·44	4·21	4·5849
24	**3·98	**2·36	0·73	1·2261
25	1·95	1·99	2·40	2·3216
		(*b*) *Field No. 36/B*		
1	**16·4802	1·6750	2·5332	3·8663
2	1·9864	5·3278	2·6366	2·8126
3	2·4506	2·9278	2·9897	2·9295
4	0·7173	2·2528	2·7870	2·5295
5	4·0136	3·0861	3·5972	3·5931
6	4·9334	4·0945	3·4374	3·6534
7	2·6667	3·2778	2·7502	2·7890
8	5·1556	2·3445	3·1225	3·2579
9	**6·2963	2·8778	1·7297	2·2946
10	1·8086	2·8500	3·2978	3·1068
11	1·2642	*3·7361	1·6282	1·7808
12	3·0383	2·2750	2·9634	2·9090
13	2·7358	2·1195	1·9499	2·0445
14	4·0259	*5·0000	2·2317	2·6618
15	3·0630	4·9278	2·7520	2·9789
16	3·5309	6·3528	3·1339	3·4632
17	1·0568	3·2278	2·1292	2·1194
18	3·5506	*5·2445	2·1396	2·5613
19	2·3716	*5·8528	2·2357	2·5744
20	**4·2963	**5·1945	1·2130	1·8827
21	4·3951	4·0695	2·3535	2·7141

** Indicate significance at one per cent.
* Indicates significance at five per cent.

APPENDIX II—*concld.*

Serial No. of the plot	Row (9)	Column (8)	Error (72)	Total (89)
		(b) *Field No. 36/B*—contd		
22	3·1914	4·1611	4·5473	4·3752
23	*4·6284	**5·6028	1·9424	2·5430
24	5·1963	*6·0445	2·6742	3·2321
25	1·7086	7·2000	4·0367	4·0854
		(c) *Field No. 35/B*		
1	1·0667	1·8778	1·1833	1·2340
2	1·8222	0·7972	1·5750	1·5301
3	1·7827	1·8778	1·7049	1·7283
4	3·8469	3·5861	2·1108	2·4190
5	0·7605	**3·1750	1·1411	1·2854
6	1·7580	1·8111	1·8636	1·8482
7	3·2395	3·3000	3·6951	3·6135
8	*3·3086	1·1528	1·5725	2·5194
9	3·7235	2·2028	3·0207	3·0182
10	**10·0938	**2·4444	0·3549	2·4265
11	1·6840	*6·5611	3·0951	3·2639
12	*6·0605	*6·2444	2·8772	3·5017
13	2·0691	2·2278	1·9747	2·0070
14	*5·9556	3·3000	2·7444	1·9955
15	2·9630	5·0278	3·3241	3·4407
16	0·7901	3·7347	3·2332	3·0312
17	3·6247	2·3111	3·7247	2·7785
18	*4·2148	3·6167	2·0429	2·4040
19	**3·1556	**3·3445	1·1500	1·5501
20	1·8630	5·3500	3·6741	3·6416
21	6·2469	2·4528	4·0886	4·1598
22	3·6308	3·1778	3·1392	3·1924
23	4·6038	7·3863	3·6176	4·0561
24	2·9000	2·1500	3·1222	3·0124
25	2·1926	1·9000	2·8537	2·7011

**Indicate significance at one per cent.
* Indicates significance at five per cent.

SELECTED ARTICLE

METHODS OF ANALYSIS AND APPRECIATION OF WHEAT, FLOURS AND BREAD, PARTICULARLY IN RESPECT OF INVESTIGATIONS ON THE BAKING VALUE

(SECOND PART)

BY

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CHAPTER II

ANALYSIS OF FLOURS

The quality of the flour is the fundamental basis of good bread and it cannot be estimated in relation to a single factor only. The data that must be taken into consideration when judging whether a flour is suitable for bread-making are many and complex. Bread is in no sense an inert, but rather a living organism, in which are to be found numerous chemical and bio-chemical phenomena that, as will be seen later, have an important influence on its quality.

The tests to which flours must be submitted are the following: (A) Physical and organoleptic—(B) Chemical—(C) Bio-chemical—(D) Physico-chemical—(E) Mechanical.

Before discussing the methods of analysis, it is necessary to mention briefly the taking and preparation of samples.

Taking of samples.—This operation should be carried out with care and accuracy in order to obtain a homogeneous sample which corresponds with the flour from which it is taken. Special instruments, called sampling rods, allow material to be taken at varying depths, either from the sack or in the warehouse in which the flour is stored.

The samples are then mixed together according to the methods used for mixing the grain and the prepared sample is placed in a sealed tin treated with paraffin and kept until required for use.

(*A*) *Physical and organoleptic examination*

This is divided into : examination by touch (to ascertain the degree of softness of the flour, whether it contains lumps, etc.)—examination of the odour—examination of the taste—examination of the colour—examination of the fineness.

Examination of the odour.—The odour of the flour should be the same as that of wheat. When there is any doubt, the odour may be brought out by boiling the flour in water with a small quantity of caustic soda.

Examination of the taste.—The taste should be that of wheat. If the taste is unpleasant or abnormal, the flour has either not been properly preserved or else contains some foreign substance.

Examination of the colour.—This is carried out to a large extent both in the milling industry and in the trade. The colour of the flour gives a fairly accurate idea of quality both as regards bolting and the presence of foreign bodies which affect its purity. The determination of the colour of the flour may be divided into : determination of whiteness (reflected colour)—determination of the chemical constituents which give colour to the flour.

The determination of colour by reflection does not clearly indicate the content in chemical elements which give colour to flours. The factors which determine the greatest differences in colour are as follows : (1) content in bran—(2) content in carotin—(3) presence of impurities and foreign bodies, such as dust, weed seeds, rust, spores, etc.

The content in bran depends on the system of milling employed : the content in carotin on the variety of wheat milled. The variations in colour, due to foreign substances, are chiefly dependent on the colour of such substances, but may also be due to other causes, such as the oiliness of seeds in the flour, which cause variations in the absorption of light. According to some authorities enzymatic activity, particularly that of oxidizing ferments, may also influence the colour of the flours. In addition to these factors there are others among which may be mentioned : (1) the coarseness of the grains—(2) the moisture content—(3) the degree of pressure exercised—(4) the intensity and direction of the light under which the examination is made.

It is, therefore, evident that in determining the whiteness of the flour there are many possible causes of error. The Pekar method, however, has remained the basic process followed for a rapid determination of colour in the practice both of the flour industry and of the flour trade (*i*)*.

Pekar method.—On a black smooth wooden board about 12 cm. long by about 8 cm. wide, small four-sided blocks of flour are made by means of stamps (5 cm. long, 3 cm. wide, 5 mm. thick). The board is then carefully placed at a slanting angle in a basin of hot water and left for about one minute ; it is then slowly

* The figures in parenthesis refer to the explanatory and bibliographical notes pp. 1150—1152.

removed and dried at 100° and the difference in colour is noted, which is much more apparent when the flour is completely dry.

Examination of the colour of flours.—Numerous methods are used which, according to the quantity and quality of the solvent used, lead to different and divergent results.

For researches on the colour (carotin) the Kent Jones method (2) has been adopted. For this there are required :—

(1) a Duboscq colorimeter : better results are obtained with the Pulfrich photometer and colorimeter with photo-electric cells (Lange apparatus),

(2) reagents : (*a*) benzine ; density, 0·720-730 ;

(*b*) a coloured solution obtained by using the following mixture : 10 c.c. of solution of potassium chromate at 0·5 per cent, 1·5 c.c. of solution of nitrate of cobalt anhydride at 10 per cent of solution. The whole is brought to 100° with distilled water.

The procedure is as follows : 20 grm. of flour is placed in a graduated tube, 100 c.c. of benzine is added and the whole is mixed and shaken in an agitator for one hour, after which it is left to stand for a night. The liquid is then separated from the precipitate, filtered (using filter No. 601-602) and the clear solution is compared with the sample.

Determination of fineness.—The sieving test may be carried out by hand by means of a series of small sieves with varying fineness of mesh. At the present time small electric " plansichters " are used, which answer the purpose perfectly and allow the various degrees of fineness of the flours tested to be studied.

(*B*) *Chemical methods*

Chemical tests comprise the following determinations moisture—ash—cellulose—protein substances—fats—gluten—sugars—acidity.

The determination of moisture, ash, fats and protein substances is carried out as for wheat.

Determination of cellulose.—The Weenden method adopted for wheat is also highly suitable for flours. Mention should also be made of the recently proposed Kurschner and Hanack method (3), introduced by Bellucci (4) for his experiments on flour, which is highly recommended for its rapidity and accuracy.

In a glass sphere, capacity 100 c.c., to which is attached a glass tube 1 metre long and 5 mm. in diameter, is placed a liquid reagent thus composed : 45 c.c. of acetic acid at 1·80 per cent in weight and 4·5 c.c. of concentrated nitric acid (p.s.—1·4) if the sample examined is flour or forage meal ; 30 c.c. of acetic acid and 3 c.c. of nitric acid for other by-products. By means of funnel 3 grm. of baking flour are introduced (2 grm. of forage meal or 0·3 grm. of other by-products), the refrigerating tube is applied and heating takes place by means of a small flame

taking care to avoid carbonisation of the substance. The flame is regulated and the heating at low boiling point is carried out for 25 minutes in such a way as to avoid the escape of steam from the refrigerating tube. Filtration is then carried out on a porous crucible. The glass sphere is washed with 5 c.c. of hot reagent, then with hot water, a little alcohol, ether and finally with hot water again. The crucible is placed in an oven at 105° for one hour and then weighed. It is then calcinated and weighed again. The difference between the two weights gives the value of the crude ash-free cellulose.

Determination of gluten.—The most important chemical tests are those made on the gluten and its constituents, as the baking value of the flour is chiefly dependent on this substance.

Gluten is formed by the action of water. In the cells of the wheat there are two gluten forming proteids which, under the action of water, are transformed into gluten. Gluten is considered to be formed by gliadin and glutenin and is not a simple mixture, but, according to Bugenberg de Jong, is a colloid complex, based on an electric relation between the two substances. It is evident that in extracting the gluten many variations may occur due to the system of extraction adopted and also to the physico-chemical conditions.

The content in gluten of a wheat and its qualities are clearly not only dependent on the quantity of nitrogenous substances and consequently on all the factors already described having an influence on the wheat (variety, conditions of cultivation, soil, climate, etc.), but are also influenced by other factors, among which should be mentioned the fineness of the milling and the conditions under which the gluten is extracted, such as the hardness and composition of the water employed, the length of time the dough is left to stand, the temperature of the water, the length of time employed in washing the gluten, the methods of the individual operator, etc.

It is, therefore, clear that it is necessary to standardize as far as possible the methods of extraction in order to arrive at corresponding and comparable results.

Many processes are now in use ; frequently spring water is used which, having a composition that varies from place to place leads to considerable variations. Marotta and Vercillo (5) adopted in their experiments the solution of the type advised by Berliner aud Koopmann (6). This is a solution of sodium chloride free from magnesia, which, with the addition of sodium phosphate, is brought to the ionic concentration considered by Dill and Alsberg as being the best for preventing dispersion of the gliadin (7).

Extraction of gluten.—In order to eliminate any influence due to the operator or to working conditions, it has been considered advisable to abandon the system of hand extraction and to use solely mechanical means. True extraction is carried out with the Hankoczy apparatus and for freeing the gluten from the starch still present, we use our own separator which in its method of operation resembles that of the machine adopted in the industry for the preparation of gluten as well as Arpin's "Essoreuse."

The method of procedure is as follows : 50 grm. of flour are kneaded for 5 minutes with 20 c.c. of standard solution (8) and the dough obtained is left to stand for half an hour after which two morsels of dough, 20 grm. in weight, are taken and placed in the two rubber bags of the Hankoczy apparatus and double extraction is carried out so as to obtain more accurate results. During the operation the standard solution kept at 18° is slowly dropped in until the washing water is clear.

It should be noted that the gluten thus extracted is not free from starch ; in fact, if the protein content is determined (allowing that the protein substances are equal to nitrogen $\times$ 6·25) it will be seen that the gluten contains 75-87 per cent of protein substances and that the remainder consists of starch, fats, minerals, etc.

After carrying out the extraction, the two pieces of gluten previously extracted are passed through two steel cylinders revolving in opposite directions, about a tenth of a millimetre apart. This operation takes place under a jet of standard solution and is completed in 40 minutes. In this way the greater part of the starch is expelled and the results are in agreement.

In our analysis we take, as already said, the average between the two weights, which are generally equal. This weight multiplied by 5 gives the content in wet gluten of 100 parts of flour ; the dry gluten is obtained by drying the wet gluten in an oven at 105° for 12 hours, taking care to cut the surface of the gluten crosswise after half an hour in the oven so as to facilitate the evaporation of the water.

* * * * *

Is the gluten content sufficient for estimating the quality of a flour and is there any constant relation between this quantity and the baking strength ?

Undoubtedly a good flour always contains a considerable percentage of gluten (generally a limit of not less than 8 per cent is required) and the absence or insufficiency of gluten renders the flour unsuitable for baking. It should, however, be observed that, although the strength of the flour is dependent on the presence of gluten, this is not dependent only on the quantity but also on the quality of that substance which is in relation to its physical and physico-chemical properties.

(*i*) *Dosage of gliadin and glutenin*

Research work on the constituents of gluten has been the subject of numerous studies made with a view to the estimation of the baking strength of flour. Some writers are of opinion that the relation gliadin : glutenin is a sufficiently accurate criterion for estimating the quality of the flour. The methods of extraction, however, employed by them are so diverse that the results contradict each other and no final conclusions can be reached. According to Snyder (9) the percentage of gliadin is much more important than the relation gliadin : glutenin.

Fleurent (10) believes that the most satisfactory relation gliadin : glutenin is 1/3, while Sharp and Gotner, using alcohol at 70°, found for flour a relation gliadin : glutenin which varies little and approximates to unity.

Determination of gliadin.—Ten grm. of flour are added to 100 c.c. of alcohol at 70° in a glass tube, with a roughened stopper, shaken frequently during the day. It is then left to stand for a night and shaken again in the morning, and filtered, after the flour has been deposited. The total nitrogen in 50 c.c. of the filtrate is ascertained by evaporation with a few drops of phospho-sulphuric acid adding the remainder of the acid when evaporation is almost complete and following the method of Kjeldahl as for the nitrogen test. By means of the percentage of nitrogen found, the gliadin is ascertained by multiplying by 6·25 and brought into relation with the total nitrogen substance.

Determination of glutenin.—When the percentage of gliadin is known the content in glutenin may be calculated by difference. Certain investigators prefer to determine the gluten directly and, among the methods employed mention should be made of that of Blish and Sandstedt (11).

The Blish and Sandstedt method.—Requires the following reagents:—

(1) Solution *N* of NaOH.

(2) Solution *N*/5 of HCl.

(3) Solution of Bromothymol blue (0·10 of di-bromothymol-sulphonaphthaleina are ground with 3·2 c.c. of solution *N*/5 of NaOH and brought to 25 c.c. with ethyl alcohol at 95°. It is necessary to filter).

(4) Methyl alcohol (should contain 96-99 per cent of CH_3OH and should be free from acetone).

Eight grm. of flour are mixed in a Kohlrausch flask capacity 200 c.c., with 50 c.c. of water and then shaken vigorously, and 5 c.c. of solution *N*/20 of NaOH is introduced. The mixture is shaken every 10 minutes for one hour after which methyl alcohol is added in parts of 50 c.c. at a time, shaking sharply after each addition until the 200 c.c. mark is reached. Another 5 c.c. of methyl alcohol are added to compensate for the volume occupied by the flour; the whole is well mixed and, after the starch has fallen to the bottom, the supernatant liquid is filtered through cotton. Then 50 c.c. of this liquid is taken and introduced into an Erlenmeyer flask; a few drops of Bromothymol blue are added and the glutenin is precipitated gradually adding by means of a burette the solution *N*/5 of HCl until a light olive colour is obtained, corresponding to a *p*H of about 6·4.

After being left to stand for one hour the contents are poured into a 100 c.c. tube and centrifuged for 10 minutes. The liquid is poured off and the glutenin is removed from the tube with a little water and placed in a Kjeldahl flask. The nitrogen is then determined and the value found, multiplied by 6·25, gives the percentage of glutenin.

In the Italian flours examined by us (using the alcohol at 70° method) no relation was found, between the relation gliadin : glutenin and the baking strength. This fact confirms results obtained by other investigators, and shows the impracticability of using such systems of research for estimating the value of the flour.

* * * * *

Determination of reducing sugars.—The quantity of sugar present in the flour after milling is almost negligible. After a few weeks of rest the formation of small quantities of sugar will be noticed which are necessary for good baking.

The dosage of sugar in flour is carried out by us according to the Bertrand method (12). It requires the following reagents :—

Solution A : 40 grm. of copper sulphate crystals are dissolved in water and brought to 1000 c.c.

Solution B : 200 grm. of Seignette salt +150 grm. of sodium hydroxide are dissolved in water and brought to 1000 c.c.

Solution C : 50 grm. of iron sulphate (free from ferrous sulphate, so that the permanganate of potash is not reduced) are dissolved in 200 c.c. of concentrated sulphuric acid and brought to 1000 c.c.

Solution D : Solution exactly $N/10$ of permanganate of potash, so that 1 c.c. of solution corresponds to 6·36 mgm. of copper.

The method of operating is very simple ; 20 c.c. of sugar solution obtained after clarifying the flour (see page 545) are treated with 20 c.c. of reducing solution (10 c.c. of solution A+10 c.c. of solution B) and boiled for 3 minutes, after which the glass is removed from the flame and the cuprous oxide precipitated (the solution left over after precipitation should remain blue) and filtered through a Gooch crucible or porous glass. After having filtered the blue liquid, the precipitate is placed on the filter and washed with water, until no traces of copper sulphate remain. The precipitate is then treated with 20 c.c. of solution C (iron sulphate) and dissolved forming a greenish solution. The glass and the crucible are washed with distilled water and the whole is collected in a flask and titrated with the solution of permanganate of potash.

The reactions take place as follows :—

(1) $Cu_2O+Fe_2(SO_4)_3+H_2SO_4=2\ CuSO_4+H_2O+2FeSO_4$

(2) $10FeSO_4+2KMnO_4+8H_2SO_4=5Fe_2(SO_4)_3+K_2SO_4+MnSO_4+8H_2O.$

The proportions of glucose and of maltose corresponding to the amounts of copper obtained are shown in the annexed tables and calculated for 100 grm. of flour.

Acidity dosage.—The acidity of newly milled flour is very small and varies between pH=5·8 and pH=6·2. After a certain period of time following milling,

Table for calculating the glucose content

Glucose mgm.	Copper mgm.	Glucose mgm.	Copper mgm.	Glucose mgm.	Copper mgm.	Glucose mgm.	Copper mgm.	Glucose mgm.	Copper mgm.
10	20·4	29	57·2	48	91·8	67	124·7	86	155·6
11	22·4	30	59·1	49	93·6	68	126·4	87	157·2
12	24·3	31	60·9	50	95·4	69	128·1	88	158·8
13	26·3	32	62·8	51	97·1	70	129·8	89	160·4
14	28·3	33	64·6	52	98·9	71	131·4	90	162·0
15	30·2	34	66·5	53	100·6	72	133·1	91	163·6
16	32·2	35	68·3	54	102·3	73	134·7	92	165·2
17	34·2	36	70·1	55	104·1	74	136·3	93	166·7
18	36·2	37	72·0	56	105·8	75	137·9	94	168·3
19	38·1	38	73·8	57	107·6	76	139·6	95	169·8
20	40·1	39	75·7	58	109·3	77	141·2	96	171·4
21	42·0	40	77·5	59	111·1	78	142·8	97	173·1
22	43·9	41	79·3	60	112·8	79	144·5	98	174·6
23	45·8	42	81·1	61	114·5	80	146·1	99	176·2
24	47·7	43	82·9	62	116·2	81	147·7	100	177·8
25	49·6	44	84·7	63	117·9	82	149·3		
26	51·5	45	86·4	64	119·6	83	150·9		
27	53·4	46	88·2	65	121·3	84	152·5		
28	55·3	47	90·0	66	123·0	85	154·0		

Table for calculating the maltose content.

Maltose mgm.	Copper mgm.	Maltose mgm.	Copper mgm.	Maltose mgm.	Copper mgm.	Maltose mgm.	Copper mgm.	Maltose mgm.	Copper mgm.
10	11·2	29	32·2	48	52·8	67	73·3	86	93·7
11	12·3	30	33·3	49	53·9	68	74·3	87	94·8
12	13·4	31	34·4	50	55·0	69	75·4	88	95·8
13	14·5	32	35·5	51	56·1	70	76·5	89	96·9
14	15·6	33	36·5	52	57·1	71	77·6	90	98·0
15	16·7	34	37·6	53	58·2	72	78·6	91	99·0
16	17·8	35	38·7	54	59·3	73	79·7	92	100 1
17	18·9	36	39·8	55	60·3	74	80·8	93	101·1
18	20·0	37	40·9	56	61·4	75	81·8	94	102·2
19	21·1	38	41·9	57	62·5	76	82·9	95	103·2
20	22·2	39	43·0	58	63·5	77	84·0	96	104·2
21	23·3	40	44·1	59	64·6	78	85·1	97	105·3
22	24·4	41	45·2	60	65·7	79	86·1	98	106 3
23	25·5	42	46·3	31	66·8	80	87·2	99	107·4
24	26·6	43	47·4	62	67·9	81	88·3	100	108·4
25	27·7	44	48·5	63	68·9	82	89·4		
26	28·9	45	49·5	64	70·0	83	90·4		
27	30·0	46	50·6	65	71·1	84	91·5		
28	31·1	47	51·7	66	72·2	85	92·6		

acidity increases, particularly if the flour has a high degree of humidity and is kept in damp and insanitary surroundings. The determination of acidity in units of *p*H should be carried out by means of a potentiometric apparatus.

In the laboratories for chemical tests of flours, the acidity is determined by the ordinary methods of titration ; some adopt watery extracts of flour, others use

for extraction alcohol at various concentrations. In our laboratory the following process is used : 10 grm. of flour are placed in a graduated closely stoppered tube containing 100 c.c. of alcohol at 50° (the alcohol should be carefully neutralised before use) ; the whole is shaken for one hour, filtered and, with 50 c.c. of the filtrate, the acidity is determined with a solution of sodium hydrate or potassium hydrate *N*/20. The c.c. of ordinary alkaline solution necessary for neutralising 100 grm. of flour in the dry state express the degree of acidity.

(C) Bio-chemical methods

These methods chiefly refer to the determination of the diastasic strength of flours and the fermentation strength of the yeast.

Determination of the diastasic strength.—This determination refers to the activity of the amylase, an enzyme which works in the starch and gradually transforms it into sugar.

The presence of sugar is necessary for the life of the saccharomycetes, thus providing the possibility of obtaining a sufficient amount of carbon dioxide which is the basis for obtaining a proper leavening. The production of sugar while the flour is in store is not of great importance, but is sufficient for the needs of the saccharomycetes. The amylase slowly attacks the crude starch ; the activity of the enzymes becomes more marked during kneading and fermentation, when the saccharification of the starch is considerable.

For determining the amylase in cereals the Rumsey method (13) is most commonly used ; we have preferred to use the official process of the Moscow Research Institute for wheats and flours (1931), which varies slightly from the former method (14).

In a flask, capacity 100 c.c., 10 grm. of flour are placed and left in a thermostat at 27° for 15 minutes, then 50 c.c. of distilled water is added at a temperature of 27°. The mixture is left to stand for one hour, always at 27°, and is shaken every 15 minutes. Then 15 c.c. of solution of copper sulphate (60 grm. of $CuSO_4$) in 1000 c.c. is added ; 15 c.c. of solution of caustic sodium (12·5 grm. of NaOH) in 1000 c.c. (15) and the whole is held at a temperature of 45° to 50° for 30 minutes. It is then left to cool and brought to volume, filtered through a dry filter and the amount of sugar in the filtrate is calculated according to the Bertrand method. The diastasic strength is expressed in milligrammes of maltose per 10 grm. of flour.

For determining the true diastasic strength, it is necessary to have knowledge of the sugars already present in the flour. This may be ascertained directly, proceeding as above, but taking care that nothing remains in suspension when the mixture is placed in the thermostat. When very accurate results are required, the Moscow Institute proceeds as follows : 10 grm. of flour with 20 c.c. of alcohol at 78° contained in a flask, capacity 1000 c.c., are first heated in a bainmarie for

10 minutes, the alcohol is evaporated completely, 20 c.c. of distilled water is added and the preceding method is followed. The quantity of sugar found that corresponds with the sugar present in the flour should be subtracted from the first result.

Is the amylase of great importance as regards baking ? Is there a relation between the quantity of amylase and the baking value ? Certainly the amylase plays an important part in baking, though this should not be exaggerated. In practice, it is found that hard wheat flours—excellent for making pastes, but unsuitable for baking have a high diastasic strength superior to that of ordinary baking flours. In practice, the diastasic strength of Italian wheats and flours has proved to be generally sufficient for baking purposes and in the contrary case it would be easy, with the addition of appropriate substances, to obtain as much as might be required. It should also be mentioned that, in baking, the high temperature causes appreciable quantities of hydro-alcoholic vapours to form, which, together with the gases produced by fermentation, exercise an action of considerable importance in the phenomena of bread making.

The above mentioned and sim ilar methods cannot give very reliable results inasmuch as the determination is carried out, not with the dough, but with as suspension in which the enzymetic action is not comparable as the conditions are totally different.

It should be added that many writers still attribute great importance to the quantity of gas developed and determine the activity of the amylase indirectly, measuring the quantity of gas which develops in the dough when yeast is added. Such testing is very simple : the gas obtained in the dough, containing a given quantity of yeast (generally 1 to 2 per cent), is collected by means of ordinary volumenometers ; or is determined by means of simple gas registering apparatus at constant pressure (16).

Brabender has constructed an apparatus, called a Fermentograph, based on the principle of the hydrostatic balance, with which is determined graphically the carbon dioxide developed by a given quantity of flour kneaded with yeast and salt [Mehlprobleme, 1932, Duisberg].

Undoubtedly a deficiency of gas—which ordinarily never occurs—has an adverse effect on successful baking ; it is much more important to know the quantity of carbon dioxide that remains in the dough than the quantity developed. Bailey and Weigley, who in their experiments have used ordinary graduated tubes for measuring the volume of the dough, have determined that the dough of strong flours lose less carbon dioxide than weak flours and, on the other hand, the more carbon dioxide is retained the greater the volume in dough. L. Borasio has arrived at the same conclusions in his recent tests (17). In fact, the good quality of a flour depends on its capacity to retain the gases of fermentation.

Determination of the fermentation strength of yeast.—Up to the present experiments have been limited to determining the fermentation strength by means of the Hayduck method. In this method 40 g. of sucrose are dissolved in water and the whole is brought to 400 c.c.; 50 c.c. of this solution is taken and, using a small mortar, 10 grm. of compressed yeast are added. The whole is put in a receptacle of 500 c.c. closed with a cork and attached, by a rubber tube, to a volumenometer full of water saturated with sodium chloride so as to prevent the absorption of the carbon dioxide developed.

The determination is made in a bainmarie kept at 30° and yeast is considered good which develops 250 c.c. of gas in 90 minutes.

Other writers suggest the use of a sugar solution for determining the gas developed in the dough obtained by adding water and given quantities of yeast, although the Hayduck method is more generally used.

(D) *Physico-chemical methods*

The most important physico-chemical determinations relate to: (1) the capacity for absorbing water; (2) the degree of hydration of the gluten; (3) the degree of extensibility of the gluten.

The capacity of flour to absorb water depends on its hydrophilous constituents and is generally determined by the baker. It is advisable always to employ the same operator so as to obtain congruent data.

In order to render this determination more scientifically accurate and less subjective, Swanson and Working (18) introduced a method by which the water, left in a flour after centrifuging, is measured. The results obtained do not always correspond with reality; to obtain more accurate methods, it is necessary to employ apparatus designed for this purpose, among which should be named the electro-mechanical "Farinograph" of Hankoczy-Brabender and our "Elettroviscosigrafo", which registers graphically the consistency of a dough and, therefore, determines the degree of absorption of the flour.

In addition to these types of apparatus which function well and will be described later on, the degree of water absorption may be determined by purely mechanical means (19). The apparatus invented by us, called the "Consistenziatore", consists of a motor which drives a kneading machine, constructed for this purpose, by means of a couple of cogwheels with a worm screw. This screw, which turns on the axle of the motor in spiral grooves, is held in place by a tared spiral spring which functions as a shock absorber. To this is attached a lever which controls an index needle, varying according to the power developed by the kneading machine, and gives the exact consistency obtained. One hundred and fifty grm. are used which are kneaded, first rapidly, adding a given quantity of water (about 70 c.c.) and then more slowly, until the standard consistency is obtained. The c.c. used calculated for 100 grm. of flour give the degree of water absorption.

Degree of hydration of the gluten.—Arpin (20) has introduced a process for determining this constant. The extracted gluten is dried by means of an apparatus invented by himself and called " L'Essoreuse ". This consists of two cylinders of vulcanite which turn in opposite directions at a distance of 0·4 mm. apart. The gluten, after having been passed through 8 times, is weighed moist and then dried at 105° and the difference in weight gives the degree of hydration which is calculated as a percentage.

Determination of the extensibility of the gluten.—Berliner and Koopmann (21) have suggested a method, based on extensibility, for estimating the quality of a flour, which will now be briefly described : 2·5 grm. of gluten, extracted with a solution of NaCl at 2 per cent are divided into 70 small pieces and left for two hours and a half in a special patented flask, with 250 c.c. of lactic acid $N/50$ at a temperature of 27°. After this period the volume is read off directly from the neck of the flask. This volume in c.c. is divided by 2·5 and the result obtained is called the extensibility index and varies between 4 and 29.

Berliner has recently modified his process, working only with 1 grm. of moist gluten and 100 c.c. of solution (D. R. P. 525594).

Apart from the difficulty of this process (due to the fact that it is almost impossible to divide the gluten into so many small pieces of equal weight), the method may give data that are fairly in agreement (particularly within the extreme limits), but it has the disadvantage of limiting the investigation to the extensibility of the gluten, an important but not the only factor for estimating the baking strength of flour, since, if substitutes are added, it is impossible to indicate with such data the quality of a mixed flour.

The data which most closely approach true facts, are those obtained by mechanical methods.

(*E*) *Mechanical methods*

Mechanical methods may be divided into two categories : (1) methods based on examination of the gluten—(2) methods based on examination of the dough.

Among the instruments used for investigations of the quality of the gluten, mention should be made of the Bolland apparatus (22), known as the " Aleurometer " now in use, and the " Gluten Tester " of Liebermann [1900] ; both very inexact instruments based on the measurement of the expansion of the gluten by means of heat.

Hankoczy Farinometer (1910)

The Hungarian scientist, Hankoczy (23) was the first, to invent, in 1905, an apparatus for measuring the elasticity of gluten by means of the action of air pressure, provided by a column of mercury, on a thin layer of that substance. This apparatus was afterwards improved and introduced in 1910 for determining the physical properties of dough.

At the present time it is preferred to operate on the dough rather than on the gluten as, in addition to the difficulties of extraction, gluten extract has not the same characteristics as the gluten still contained in the dough, while the physical characteristics of dough, though for the most part dependent on the gluten, are also dependent on the presence of other component elements (starch, sugars, dextrin, protein, cellulose, etc.), all of which contributed towards the suitability or otherwise of flour for baking purposes.

Numerous kinds of apparatus have been invented, the two most important being the Chopin " Extensimeter " and the Borasio-De Rege " Pneumodynamometer ".

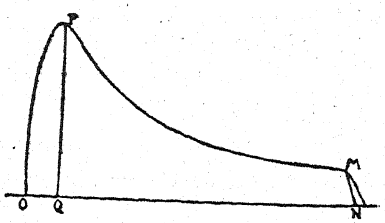

Fig. 1. Graph obtained with the " Extensimeter ".

The composition of the dough, the process of separation, the duration of kneading and of the rest period and the other manipulations should always be carried out according to the same system.

The Chopin Extensimeter is extensively employed and curves are obtained with it which have the following characteristics :—

P = tenacity, calculated by the maximum pressure registered : PQ ;

G = square root of the volume of the bubble at the moment of breaking ;

W = average working effect divided by the weight of the layer of dough.

Chopin writes that, for a flour to satisfy purchasers from the point of view of its plastic quality, the raising effect W should be as near as possible to a determined value corresponding to a given region (quality of the wheat used in that region and the system of baking employed). Thus for Paris this value is fixed as W = 100.

Chopin is of opinion that such value should not fall below a determined limit, *e.g.*, below 20 for Paris.

The apparatus works under pressure and the results are fairly good, though the speed of the escape of air is rapid and irregular, for which reason the curve has characteristics that are not absolutely exact. The determination, that is made on the basis of the volume of water reached as read off by the operator on the graduated column of the apparatus, is also subject to error on account of the rapidity of the test.

The " Pneumodynamometer " invented by Borasio and De Rege (24) is completely automatic and very accurate.

The outstanding merits differentiating the " Pneumodynamometer " from other similar apparatus, increase its practical and scientific value and depend on the fact that, in addition to the advantages and facility of a specially devised automatic device, the quantity of air is kept strictly constant and is not influenced by the different resistances of doughs made of various flours.

The curve expresses the force exercised by the air in modifying the form of the layer of dough and the area, enclosed in the curve, is called the strength of the flour.

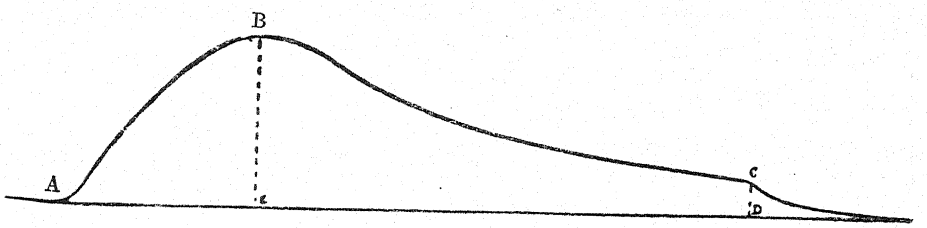

Fig. 2. Typical graphs obtained by the " Pneumodynamometer ".

From the graph traced by the " Pneumodynamometer " important indication may be obtained, allowing the quality of a flour to be estimated most completely (25). These indications are the following —

(1) Tenacity, represented by the maximum ordinate of the curve which is given by the segment B E, indicating the pressure in mm. of mercury.

(2) Elasticity, volume in c.c. of the bubble at point B.

(3) Extensibility, volume in c.c. of the bubble at the moment of breaking.

(4) Ordinate of breaking, represented by the segment D measured in mm. of mercury.

(5) Relation extensibility : Elasticity.

(6) Strength of the flour, area ABCD in c.c., enclosed by the curve.

(7) Working effect=extensibility $\times$ flour strength.

The most important data are extensibility and strength ; it should, however, be noted that flours frequently occur with slight resistance capacity and great extensibility, or on the other hand, with great resistance capacity, but deficient in extensibility.

The working effect, equivalent to the product of strength multiplied by extensibility, is in proportion to the modifying effect produced by gases acting on

the dough, and includes these characteristics, so that if one or the other is absent or even is not very marked, the effect will be *nil* or slight, and in this way the quality of a flour may be judged.

Taking the values of the index of working effect as a basis, the writers have fixed limiting values for this index, thus giving the following classification of flours :—

Inferior	500—1,500
Mediocre	1,500—3,000
Good	3,000—4,500
Superior	above 4,500

The Chopin apparatus and the Pneumodynamometer register the strength employed in dilatation and allow the modifying activity in the dough to be ascertained. This work activity is the most interesting and important from the point of view of baking as it is that which operates in the phenomena of fermentation and baking.

It certainly cannot be stated that there is any absolute agreement between the curves and the baking tests on account of the many complex phenomena, chemical, bio-chemical and physico-chemical, which arise during the process of baking ; at the same time it should be recognised that these instruments provide the most accurate method of estimating the quality of a flour. Their precision gives them a special value, both from the practical and scientific points of view, and makes it possible to study many problems regarding the properties of dough. Among these, one of the most important is that concerning the strengthening of flour, *i.e.*, a knowledge of the quantity of strong flour required in order to strengthen a weak flour. This problem may be solved by a simple calculation and, if, for obvious reasons due to the influence that certain components in the flour exercise in the mixture, accurate results are not obtained, they are at least approximately correct and provide a satisfactory criterion for estimating the percentage required (see L. Borasio, Studi sulle farine di grano, Vercelli, 1934).

(*F*) *Methods based on the measurement of the force required for kneading*

If the work of a kneader is observed, it will be seen that the energy output required in kneading differs according to the quality of the flour. On this principle are based the Farinograph of Hankoczy and Brabender, the Electroviscosigraph in our laboratory, the dynamometric kneader of Feyte and Potel and the apparatus devised by Swanson and Working.

In 1912, Hankoczy invented the Farinograph, which was improved between 1927 and 1930 and brought to its present form in collaboration with Brabender. This apparatus, which works with an electrodynamometer, registers under the form of a curve the physical characteristics of the dough and also determines the capacity for water absorption in the flour. This factor is determined in a preliminary test by adding to the flour the quantity of water necessary for obtaining a dough of a previously established consistency. According to Brabender

this determination is carried to a high degree of precision (0·2 per cent) ; a degree of sensitiveness so high actually does not exist.

Investigations on the baking quality of the flour are carried out on 300 grm. of flour at constant consistency, working, according to Hankoczy, for 15 minutes. Many factors may be obtained from the graph, though Hankoczy considers two only, namely :—

(1) the duration of rising in the dough, calculated in minutes from the beginning of the curve to the moment at which the curve no longer ascends ;

(2) weakening or fall in consistency expressed in cm^2, which is determined by measuring with a planimetre the area comprised between the ordinates corresponding on the one hand to complete rising and on the other to the ordinate of the 15th minute of kneading.

By the use of these methods, Hankoczy suggests a classification based on the duration of kneading and the fall in consistency (26).

Does, however, Hankoczy's classification correspond with the baking test and is there a relation between the curve obtained and the experimental tests ?

It cannot be denied that a relation does exist between the course of the curve and the baking value ; a relation which is sometimes uncertain in respect of certain flours and particularly in mixtures containing substances with a high power of absorption.

Hankoczy attributes this to the fact that the dough, while having a satisfactory consistency, has not sufficient elasticity and extensibility to retain the gases of fermentation and the bread obtained is, therefore, relatively small in volume.

Similar results are obtained with the dynamometer of Feyte and Potel, with the " Elettroviscosigrafo ", and with the apparatus of Swanson and Working (27). The following facts have been determined by means of the first instrument :—

(*a*) that the effort required in kneading a flour is proportional to the duration of the process ;

(*b*) that operating under the same conditions as for testing with the Extensimeter of Chopin, a proportional relation has been observed between W (work) and the force required for kneading.

Using the " Elettroviscosigrafo ", which the writer has devised for measuring the output of effort in kneading by means of a registering wattmeter, it has been possible to determine :—

(1) that the absorption of force varies according to the flours ;

(2) that there is no relation between this force and the baking value, as the relation between the baking value, and the curves traced at equal consistencies is not invariable ;

(3) that the absorption capacity is not a function of the baking quality;

(4) that as colloidal substances are involved, many factors have a considerable influence on the behaviour of the curves, such as the type of kneader employed, its speed, the temperature, the chemical substances added, etc.

All these instruments are based on the principle of the degree of working under pressure. In our opinion, this working is not quite comparable to that which the dough undergoes during fermentation and baking. In fact, it is true that in mixtures containing substances with a high capacity for absorption (rice, facula, carob, clay, gesso, etc.), the working under pressure and the consistency of the paste increase considerably and register excellent curves from every point of view, but the practical results are not satisfactory, as they do not correspond to the baking tests.

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(1) In order to facilitate the colour test special apparatus with photo-electric cells have recently been introduced which determine the light reflected by the flour.

(2) KENT JONES, *Modern Cereal Chemistry*, 1927, Liverpool.

(3) KURSCHNER and HANACK, in *Zeitschrift für Untersuchung der Levensmittel*, Berlin 1930, **59**.

(4) BELLUCCI, in *Annali di Chimica applicata*, Roma 1932, **22**.

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(6) BERLINER and KOOPMANN, in *Mitteilungen uber Lebensmitteluntersuchung*, 1929, **20**.

(7) DILL and ALBERG, in *Zeitschrift für Untersuchung der Lebensmittel*, Berlin 1930, **59**.

(8) The standard solution is obtained thus : a certain quantity of magnesium free sodium chloride at 2 per cent is prepared and used as a solvent, two concentrated solutions are prepared (for example, 4 per cent of anhydrous salt); one of bi-sodium phosphate and the other of mono-sodium phosphate and are mixed in such proportions as to obtain pH=6·8 (Bromothymol blue indicator). This solution is diluted with the solution of sodium chloride previously prepared so as to obtain a concentration 0·1 per cent of anhydrous salt. When used the solution should be kept at 18°.

(9) SNYDER, in *Journal of the Chemical Society*, 1905, p. 1068.

(10) FLEURENT, in *Comptes rendus de L'Academie des Sciences*, Paris 1896, tome **123**, p. 755.

(11) BLISH and SANDSTEDT, in Cereal Chemistry 1925, **2**; 1927, **4**.—*Journal of the Association of Official Agricultural Chemists*, Menasha (Wisconsin) 1926, **9**, p. 417.

(12) BERTRAND, in *Journal de las Societe de Chimie biologique*, Paris 1906, **35**, p. 1285.

(13) RUMSEY, in *American Institute of Baking Bulletin*, 1922.

(14) The difference between the Russian method and that of Rumsey is that: in the latter the clarification is carried out with the aid of 3 c.c. of solution of sodium tungstate at 15 per cent and 5 drops of solution of Thymol blue at 0·04 per cent, sulphuric acid being then added drop by drop until a rose colour is obtained. The acidity of the solution may be caused by the inversion of small quantities of the sucrose present in the flour, which brings about an increase in the maltose content. For this reason the Russian method, which works under alkaline conditions, gives more precise data.

(15) Clarification may also be carried out using 15 to 20 c.c. of solution of zinc sulphate at 4·5 per cent, solution of sodium hydroxide at 4 per cent, the whole being heated at 45° to 50° for 30 minutes.

(16) MARKELY and BAILEY, An automatic method for measuring gas production and expansion in doughs.—*Cereal Chemistry*, 1932, **9**.

ROY IRVIN, An improved apparatus for measuring gas production and expansion in doughs.—*Ibidem*, 1935, **2**.

(17) In order to obtain data which correspond more closely with reality, BORASIO carried out fermentation tests operating on small loaves of 100 grm. of flour which were left to ferment freely without any external influence so as to ascertain their true capacity for expansion and growth. The flour was kneaded with 2 per cent of ordinary compressed yeast of regular consistency; the volume of the loaf before fermentation is carried out in a graduated tube with the addition of water; the quantity of gas developed is determined with ordinary volumenometers; the volume reached by the loaf is measured approximately with volumenometers which function with the displacement of liquid. The baking ovens are kept at 30°. (See L. BORASIO *Studi sulle farine di grano*, Vercelli, 1934).

(18) SWANSON and WORKING, in *Cereal Chemistry*, 1933, **10**.

(19) See for the apparatus invented by these writers, *Ibidem*, 1933, **10**.

(20) ARPIN, *Farines, fecules et amidons*, Paris, 1913.

(21) BERLINER and KOOPMANN, in *Zeitschrift für das gesamte Muhlewesen*, 1929.

(22) BOLAND, in *Revue des Services de l'Intendance*, Paris, 1888.

(23) HANKOCZY, in *Molnarok* Lapja, Budapest, 1905.

(24) BORASIO, L. and DE REGE, F., in *Giornale di Risicoltura*, Vercelli, 1932 et 1933.

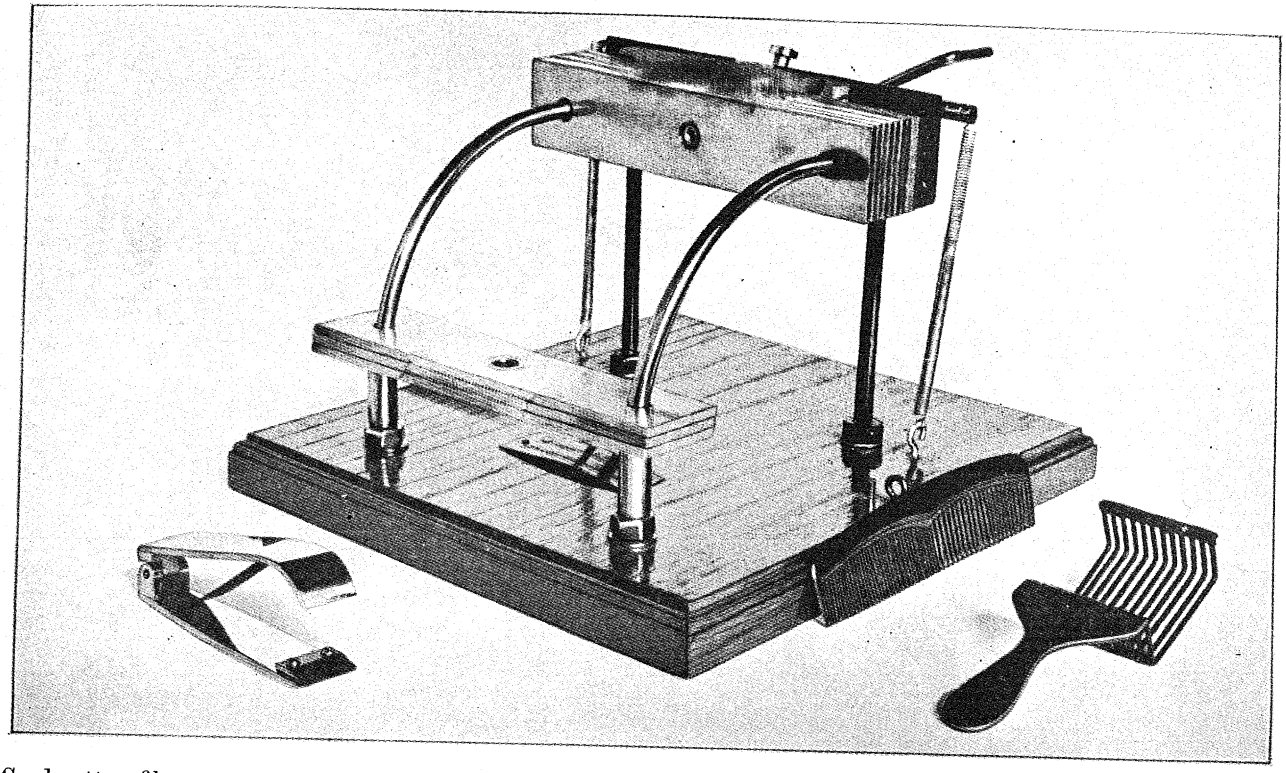
(25) Id., Id., *Istruzioni per l'impiego dell' apparecchio*, Vercelli, 1934.

(26) Scale showing the numerical values of the curves, as adoped by Hankoczy—

Length of kneading process	Points	Fall in consistency	Points
10 minutes	100	0 cm²	100
1 minute	1	60 cm²	0

The two points, found for every flour, are added together, divided by 2 and the result gives the numerical value. Hankoczy, for studying exhaustively the properties of a flour, determined with the Farinograph the consistency of a dough containing salt and yeast in given percentages and kneading took place alternating with periods of fermentation. The curve obtained was compared with that of the development of the CO_2 obtained with the Fermentograph. According to the writer the data obtained with the Farinograph are thus integrated and more accurate results are obtained. (See *Mehlprobleme*, 1932, Duisburg).

(27) The SWANSON-WORKING apparatus is merely a small standard kneader to which is attached a registering wattmeter or a dynamometer apparatus. The investigations on the determination of the degree of water absorption have given satisfactory results particularly with the completely mechanical apparatus.



Seed-cotton-fiber sorter, showing mechanical features of construction with three combs in resting position, together with comb, forceps, and pusher.

[Reproduced from the *United States Department of Agriculture Circular No.* 360.]

ABSTRACT

A device for separating different lengths of fibres from seed cotton. HOMER C. MCNAMARA and ROBERT T. STUTTS (*United States Department of Agriculture Circular No.* **360**, *August 1935*).

[With Plates LXIV & LXV]

SUMMARY

A simple device for determining the variation in fibre-length on a single seed of cotton would greatly help the cotton breeder as it would enable him to evolve strains which possess greater uniformity in length.

The device (Plates LXIV & LXV) described in this circular consists of a set of brass combs each $\frac{1}{16}$ in. or $\frac{1}{8}$ in. thick mounted side by side on two guide rods, which curve down to the base plate. The combs are held in position by a screw by turning which each comb is successively released and glides down the guide rods on the base. A metallic bar with an adjustable spring tension serves to hold the combed seed in a cavity provided for it behind the combs. The authors recommend combs having 24 teeth to an inch, each tooth being $\frac{1}{64}$ in. in diameter as being the most suitable. The accessories required are a spare comb, forceps and a wire depressor for slipping the fibres into the combs.

In performing a test, the seed is combed into a " butterfly " taking good care to remove or break as few fibres as possible in the combing process. The combed seed is placed in the cavity provided for it in such a manner that the fibres on one side lie at right angles to the combs. These fibres are pressed down with the wire depressor and the seed is held in position by the tension bar. A few combs are released until the ends of the longest fibres just project beyond the combs. These are gripped with a tweezer, pulled out and laid on a velvet plush. Another comb is released and the operation is continued until all the fibres on one side of the seed are extracted and laid on the plush. This will enable the worker either to form the complete array or to estimate the percentage of fibres above a certain length.

Details of some experiments conducted for the purpose of determining the most suitable size and spacing of the teeth of the comb are given with photographs.

The authors found that the amount of hairs removed during the preliminary combing process depended upon the tester and showed a tendency to increase with the longer staple. Further, the combed hairs contain fibres of all lengths borne on the seed, but among these there is an excess of shorter fibres.

The chief sources of error discussed are (1) while the fibres are assumed to be aligned at the basal end, they are actually distributed over the curved surface of the seed ; consequently the base of each group length extracted will be uneven, (2) there will be a slight curling of the fibre ends when gripped by the forceps.

A transparent sheet of celluloid with a series of horizontal lines one-sixteenth of an inch apart from a common base line serves to measure the individual group length.

ABSTRACTOR'S REMARKS

Uniformity in fibre-length is a valuable asset and any simple device which would help the cotton breeder to choose a more uniform variety should be welcome.

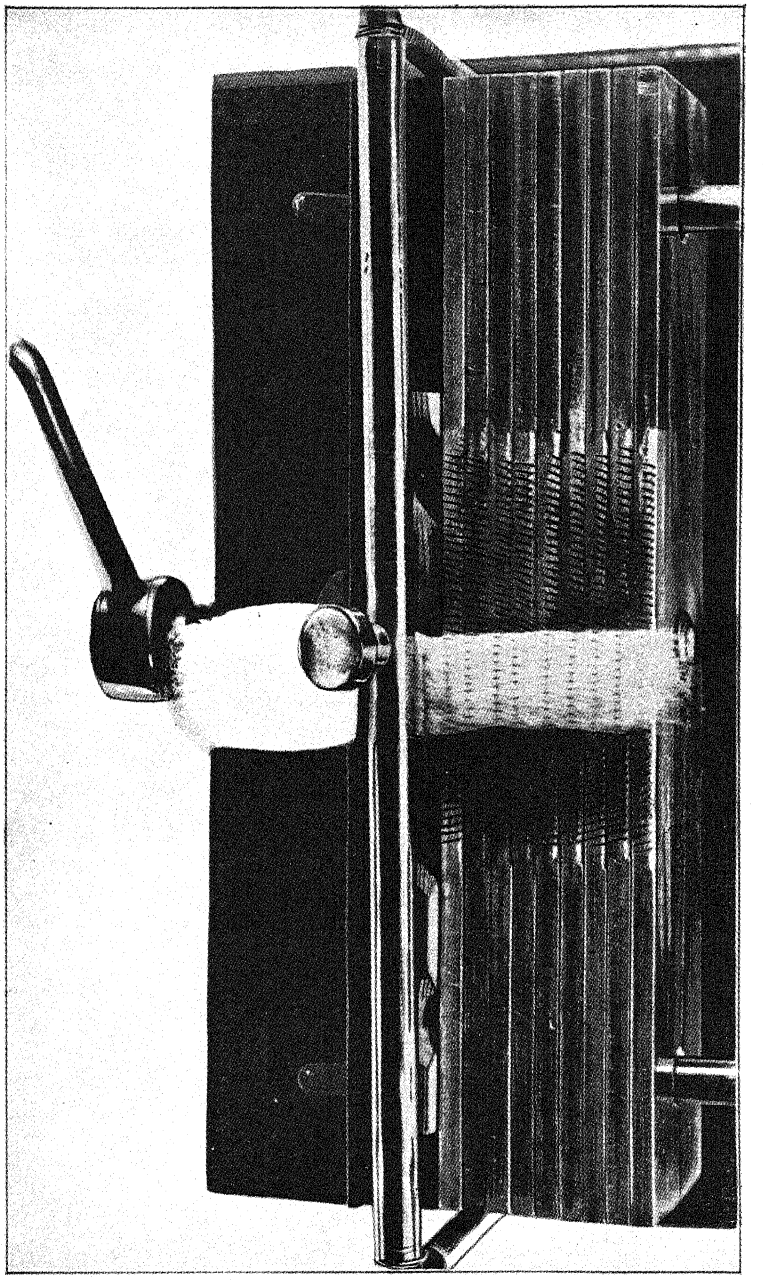
In the present case, in addition to the source of error discussed by the authors, there is one which has apparently been overlooked by them. If the cotton fibre is nipped at the ends and stretched, in most cases it will break at or near the apical end where the fibre tapers and is consequently weak. In an investigation* carried out at this Laboratory it was found that nearly 80 per cent of the fibres broke near the apical end. In view of this the usual practice in removing fibres from the seed is to grip them as near the seed-surface as possible. In the method under review, after the seed is placed behind the combs the fibres are nipped in the tweezer at their apical end and pulled. It is highly probable that in this process some of the fibres will break and consequently the array obtained will not be truly representative. It would have been highly useful if the authors had given comparative results for the same cottons as arrayed by the present method and by an older method.

In this connection it should be noted that the number of hairs which might break on pulling near the apical ends will vary with the variety, as it will depend upon the tenacity with which the fibres are held by the seed. Hence, this error will be variable, and not constant, for the different varieties.

The authors state that in the combing waste there was an excess of shorter fibres, resulting in lowering the mean fibre-length of this waste. In the experiments performed at this Laboratory to which a reference has been made above, no such effect was observed. This lack of agreement may either be due to the peculiarity of the cottons concerned, or to the difference in the amount of force applied in disentangling the fibres. The latter explanation is more likely as the authors removed from 12·8 to 22·3 per cent fibres in the combing process as against 7 per cent to 17 per cent in the experiments performed at this Laboratory.

Finally, it should be mentioned that owing to the variation from seed to seed, it would be necessary to test a moderately large number of seeds to avoid large sampling error. (NAZIR AHMAD).

* Variation in the Properties of Cotton Fibre in relation to its Position on the Surface of the Seed, by R. S. Koshal and Nazir Ahmad, *Ind. Cent. Cot. Com., Tech. Bull., Series B*, No. 14.



Close-up view of seed-cotton-fiber sorter.
[Reproduced from *United States Department of Agriculture Circular No.* 360.]

REVIEW

Plant Breeding Abstracts Supplement II: Summary of Reports received from stations in the British Empire, 1932-35. [Compiled by the Imperial Bureau of Plant Genetics (for Crops other than Herbage), School of Agriculture, Cambridge.] 63 pp. Price 5*s*.

This Supplement to Plant Breeding Abstracts which has been compiled from the annual reports received at the Imperial Bureau of Plant Genetics, Cambridge, is intended to give a concise account of the work on plant breeding and related topics in the British Empire during 1932-35 and one which will be at the same time in a convenient form for reference. The amount and range of plant breeding work in the British Empire is of course enormous and the publication has involved the scrutiny of over 400 reports.

Much of the work referred to is only available in annual reports, being more or less of a routine nature, and but for the activity of the Imperial Bureau of Plant Genetics would have remained inaccessible to the general reader. Another valuable feature of the Supplement is that many of the items included concern work which though likely to be published in the future has not yet reached that stage.

The crops dealt with cover practically the whole range of economic plants with the exception of herbage plants and the work reported includes breeding, genetics and cytology. There is also a section on the genetics of plant parasites.

The Supplement is very comprehensive and is planned in such a way that the work on a given crop in a given country can be turned up at once; an extensive index is also provided.

Reviews of this kind are invaluable to practical breeders in giving a survey of the work in progress in the whole field of plant breeding and should be read with interest also by all those interested in modern genetics and its application to agricultural plants.

A similar supplement is in process of compilation from the reports received from foreign stations.

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ERRATA

The Indian Journal of Agricultural Science, Vol. VI, Part II, April 1936.

Page 189, line 10 from bottom, *for* 'Local' *read* 'Locust'.

Page 190, heading 2, *for* *'Schistocerca Gregaria'* *read* *'Schistocerca gregaria'*.

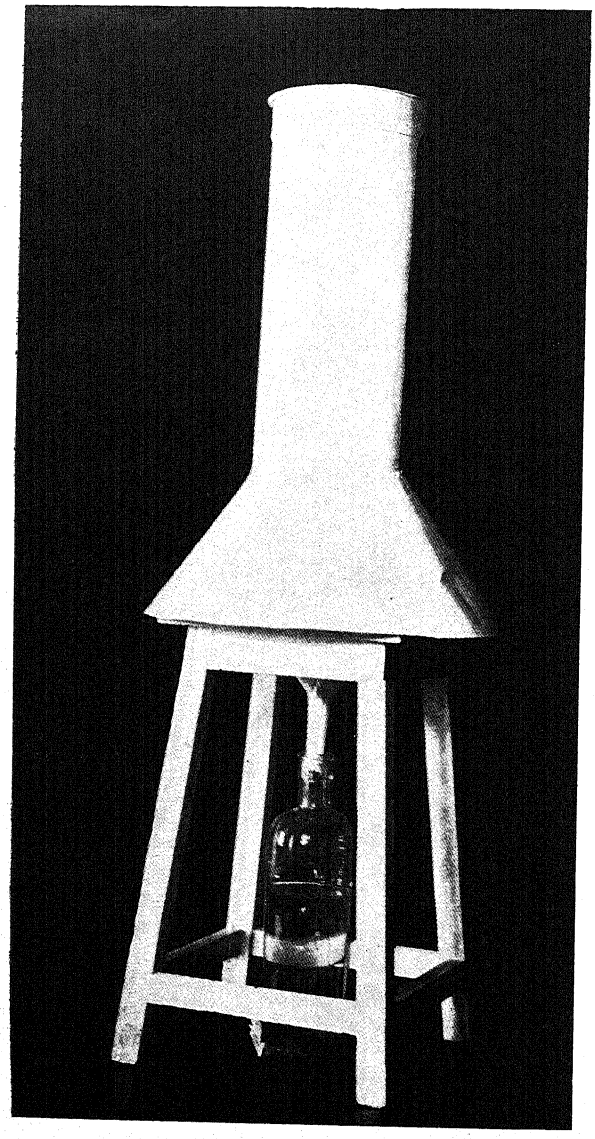
Page 195, heading of Table IV, *for* *'28-32 C'* *read* *'28-32° C'*.

Page 196, line 3, *for* *'rythm'* *read* 'rhythm'.

Page 207, line 4, *for* 'sandy-loan' *read* 'sandy-loam'.

Page 218, line 3 from bottom, *for* 'eigh thours' *read* 'eight hours'.

Page 263, footnote, line 3 from bottom, *for* 'It is implied' *read* 'It is not implied'.



Portable drain-gauge.

ORIGINAL ARTICLES

AGRICULTURAL METEOROLOGY

STUDIES ON SOIL-MOISTURE IN RELATION TO MOISTURE IN THE SURFACE LAYERS OF THE ATMOSPHERE DURING THE CLEAR SEASON AT POONA

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(Received for publication on 25th May 1936)

(With Plate LXVI and ten text-figures)

INTRODUCTION

In a previous communication* [Ramdas and Katti, 1934,[1]] the phenomenon of the evaporation of moisture from a soil surface containing only hygroscopic water and the reverse phenomenon during night, *viz.*, the absorption of moisture from the air layers near the ground by the desiccated soil surface, were shown to exert a controlling influence on the distribution of moisture with height in the air layers. From observations of the moisture contents of the soil surface and of the air at different levels above ground it was found that, during the "evaporating regime" (during day), the pressure of water vapour decreased with height, whereas, during the "absorbing regime" (at night), the vapour pressure increased with height. In the preliminary studies which have been discussed in the above paper the observations were taken only twice daily with the local soil, *viz.*, at sunrise and at sunset. With a view to study the diurnal variation in the moisture content two-hourly measurements were made with representative soils from different parts of the country, the soils being exposed under identical conditions in the Agricultural Meteorological Observatory at Poona. Such measurements were conducted during the summer of 1934 as well as during the winter of 1935. Besides these, measurements of the moisture content of the surface layers of the soil in the bare ground of the Agricultural Meteorological Observatory were also made in 1935. Experiments on the absorption of moisture by dry soils and measurements of various related physical properties of typical soils were also made in the laboratory about the same time. In all these investigations the meteorological aspect has been kept in the fore-front and the soils have been used as they occur, *i.e.*, without separating them into their components by mechanical or chemical analysis. These results are discussed in the following sections :

* "Preliminary studies on soil-moisture in relation to moisture in the surface layers of the atmosphere during the clear season at Poona" by Ramdas, L. A. and Katti, M. S., *Indian Journal of Agricultural Science*, Vol. IV, part 6, 1934. (This paper will be referred to as paper 1 in the following pages.)

Section I

Experiments on the evaporation and absorption of water vapour by different soils

1. Two-hourly observations of the moisture contents of a few soils during the summer of 1934 (23rd May to 2nd June 1934)

After realising from the preliminary experiments discussed in paper 1 that the results indicated an interesting line of work, steps were taken (1) to pursue work with different types of soils and (2) to commence observations several times daily so as to study the diurnal variation in the moisture contents in greater detail. Although the season was well advanced and the simple climate of winter had given place to one of the sea-breeze type, there was a spell of clear weather and it was considered worth while to start comparative observations in order to see how, under similar meteorological conditions, soils differed from one another.

The observations were begun at the Agricultural Meteorological Observatory with Poona black cotton soil at 0600 hrs. on the 23rd May 1934 ; on the morning of the 25th, samples of alluvial soil from Sakrand (in Sind) were added ; on the morning of the 1st June 1934, samples of quartz powder, brown Dharwar schist and red laterite were also included. The two-hourly observations on the soil samples were continued up to 0600 hrs. on the 2nd June after which they were discontinued owing to the onset of cloudy weather.

During the above period (23rd May to 2nd June) the skies were clear but the simple climate of winter had changed to one of the " afternoon sea-breeze " type. Ordinarily, during calm weather in winter, the air sample over Poona does not change materially during the day or, often, during a series of days. The convective winds during such days cause mixing of the air layers in the vertical direction. In February and later months of the clear season the westerly sea-breeze begins to arrive at Poona about sunset. The time of arrival becomes earlier as the season advances. This breeze which replaces the land air has a higher moisture content. The sea-breeze penetrates inland slowly, dies down by midnight and later remains stagnant over the country until, after sun-rise on the next morning, the diurnal convection sets in and mixes up the shallow layer of moist air with the reservoir of dry air at the higher levels. During the sea-breeze season, *viz.*, part of February, March, April and May, the dry land air holds its sway during day time but is regularly displaced by sea-air during the night. The effect of this is seen in the psychrometric data taken at the maximum and minimum temperature epochs. These show higher vapour pressures during the night and until the next morning up to about 8 A.M., than during the day time after the convective winds have set in. During the earlier months of the season which are free from sea-breeze the mean vapour pressure is practically the same day and night. As already explained in paper 1, both during the non-sea-breeze and the sea-breeze portions of the clear season the vapour pressure increases with height during the night and decreases with height during the day.

TABLE I

Time	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700
Weight of Poona soil in grms. (Mean for 23-31 May 1934).	60·751	60·676	60·554	60·355	60·017	59·709	59·370	59·159	59·021	58·961	58·973	59·038
Weight of Sakrand soil in grms. (Mean for 25-31 May 1934).	60·435	60·379	60·321	60·256	60·197	60·144	60·104	60·074	60·053	60·041	60·034	60·043
Temperature °C. (Soil surface) . . .	22·5	27·5	35·1	44·1	52·0	57·5	60·5	61·1	59·4	56·2	51·6	45·8
Air temperature (0·3″) °C.	22·6	25·0	27·9	31·8	35·6	38·5	40·8	42·3	42·9	42·7	41·7	38·6
Air temperature °C. (one ft.) . . .	22·9	24·7	27·2	30·2	33·4	35·4	36·9	38·0	38·6	38·6	38·0	36·4
Wind speed at 1′-6″ (miles per hour) . .	4·7	5·2	5·8	6·2	6·9	7·4	8·1	8·9	8·9	8·9	9·1	9·2
Vapour pressure (0·3″) in mm. of Hg. . .	14·5	15·2	15·6	15·4	14·7	14·3	13·3	13·3	12·2	11·6	11·3	12·2
Vapour pressure (4 ft.) in mm. of Hg. . .	14·6	14·7	14·6	14·1	13·5	13·0	12·3	11·4	10·6	10·5	10·5	11·6
Humidity per cent (0·3″)	71	64	56	43	34	28	23	21	19	18	20	24

Time	1800	1900	2000	2100	2200	2300	2400	0100	0200	0300	0400	0500
Weight of Poona soil in grms. (Mean for 23-31 May 1934).	59·185	59·383	59·621	59·837	60·003	60·148	60·265	60·379	60·475	60·557	60·631	60·693
Weight of Sakrand soil in grms. (Mean for 25-31 May 1934).	60·061	60·089	60·123	60·168	60·199	60·226	60·253	60·276	60·301	60·321	60·342	60·361
Temperature °C (Soil surface) . . .	39·6	34·4	31·8	29·9	28·4	27·1	26·3	25·5	24·7	24·0	23·4	22·9
Air temperature (0·3″) °C.	34·4	31·6	29·1	27·4	26·1	25·4	24·8	24·4	24·2	23·7	23·2	22·9
Air temperature °C. (one ft.) . . .	33·6	31·1	28·8	27·2	26·0	25·3	24·8	24·4	24·1	23·8	23·5	23·3
Wind speed at 1′-6″ (miles per hour) . .	9·3	8·9	8·1	7·3	6·7	6·4	5·7	4·9	5·1	5·0	4·8	4·4
Vapour pressure (0·3″) in mm. of Hg. . .	13·3	14·1	14·6	15·3	15·6	15·4	15·0	15·2	15·2	15·2	15·2	15·2
Vapour pressure (4 ft.) in mm. of Hg. . .	13·0	13·8	15·0	15·9	16·3	16·1	15·9	15·8	15·8	15·6	15·4	15·4
Humidity per cent (0·3″)	32	40	48	56	61	63	64	66	67	69	72	70

The soil samples, weighing about fifty grammes each, were exposed in small aluminium vessels having a cross-section of 30 sq. cm. The vessels were embedded in the ground so as to bring the level of the soils inside flush with that of the soil outside. In the case of the soils of Poona and Sakrand, the samples were exposed in duplicate ; only one sample each of the other soils was used. The two-hourly observations consisted of the following measurements :—

(*a*) the weight of each soil sample (the vessels were kept tightly covered during weighing which was done quickly on a good physical balance),

(*b*) air temperature, humidity and vapour pressure at several levels starting from the soil surface (with an Assmann psychrometer),

(*c*) wind speeds at $1\frac{1}{2}$ ft. and 6 ft.,

(*d*) soil temperature at the surface of the soil, and

(*e*) the intensity of the total radiation from the sun and the sky from the records of the Moll solarigraph.

In studying these data it was convenient to plot the observations against the actual times at which they were made and from these curves to read off the hourly values. Table 1 gives the mean hourly weights of the Poona soil (mean for the period 23rd to the 31st May 1934) and the Sakrand soil (mean for the period 25th to the 31st May 1934). The hourly variations shown by the duplicate samples agree very closely and the figures in the table are the averages obtained from the duplicate samples. The mean hourly data of the following meteorological factors are also given in Table I :—

(*i*) soil-surface temperature in °C.,

(*ii*) air temperature at 0·3″ above the ground,

(*iii*) air temperature at 1 ft. above the ground,

(*iv*) the percentage humidity at 0·3 in. and the pressure of water vapour in mm. of Hg. of the air at 0·3″ and 4 ft. above the ground, and

(*v*) the velocity of wind at 1 ft. 6 in. above the ground (in miles per hour).

The hourly data bring out the characteristics of the phenomena under consideration in much greater detail than the data recorded at sunrise and at sunset and given in Table VII of paper 1. It may be noted that the soils have their *minimum* moisture content about the epoch of maximum temperature and their *maximum* moisture content at the epoch of minimum temperature. The results are easier to visualise when represented diagrammatically (Fig. 1). The epochs of *maximum air temperature, minimum saturation* of air with water vapour (humidity per cent) and *minimum water vapour content* in the air (this would be maximum water vapour content during days of no sea-breeze, *e.g.*, December, January, when we deal with the same air samples) occur practically at the same time as the epoch of *minimum* moisture content of the soil samples. During months with no sea-breeze the maximum wind velocity would also have occurred about the same time.

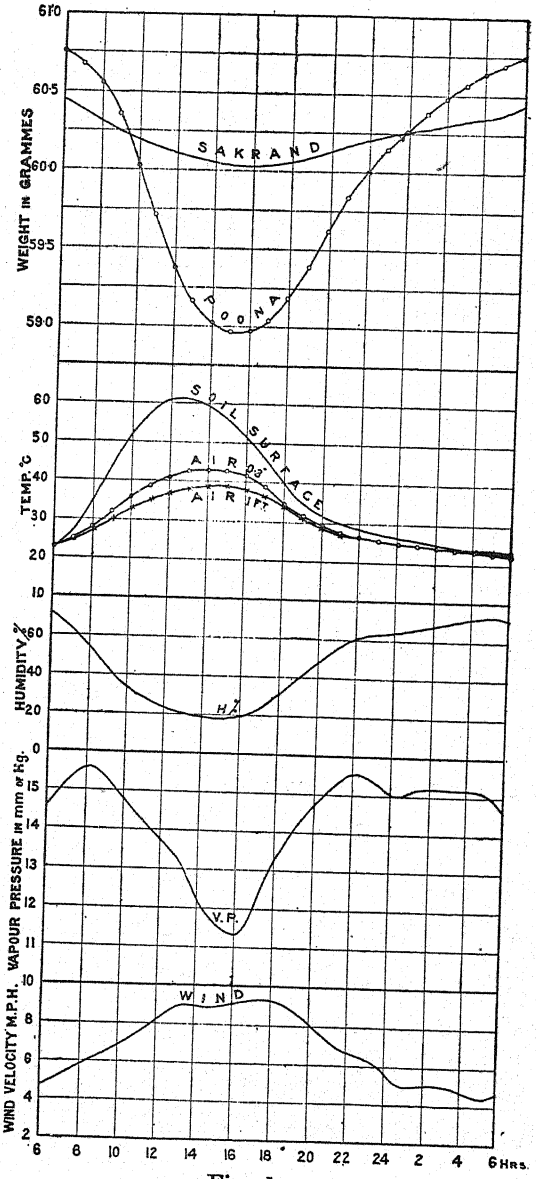

WEIGHT IN GRAMMES
SAKRAND
POONA
TEMP. C
SOIL SURFACE
AIR 9 A.
AIR 1 P.
HUMIDITY %
H %
VAPOUR PRESSURE IN mm or Hg.
V.P.
WIND VELOCITY M.P.H.
WIND
6 8 10 12 14 16 18 20 22 24 2 4 6 Hrs.

Fig. 1.

The epoch of *maximum moisture content* of the soil samples coincides with the epoch of minimum temperature and maximum saturation of the air with water vapour. As already pointed out, the air layer nearest to the ground would have a smaller water vapour content than the layers higher up in the early morning, but this is somewhat masked by the disturbance due to sea-breeze during the night. It is significant that the variation of moisture content of the soil is in phase with the variation of the air temperature and humidity percentage rather than the soil surface temperature (which closely follows the altitude of the sun and has a maximum value when the sun is over-head).

The other interesting fact which may be observed from Fig. 1 is that the diurnal variation of moisture in the Poona soil is more than four times that of the soil from Sakrand [Ramdas and Katti, 1934,[2]]. The very first day's results suggested that each soil type may show a characteristic diurnal variation. This expectation has been borne out by the observations recorded in June 1934 (0600 hrs. of 1st June 1934 to 0600 hrs. of 2nd June 1934) and the later observations made during the clear season October 1934 to May 1935. Fig. 2 shows the hourly variation of the moisture contents of soils from Poona, Dharwar and Sakrand, a sample of red laterite and a sample of quartz powder respectively from 0600 hrs. of 1st June 1934 to 0600 hrs. of 2nd June 1934. The sample of quartz powder did not show any variation of weight whereas the other soils showed different diurnal variations in their weights. The maximum weight of the soil (in the morning) when its moisture content is maximum and the minimum weight (in the afternoon) when its moisture content is minimum are given in Table II.

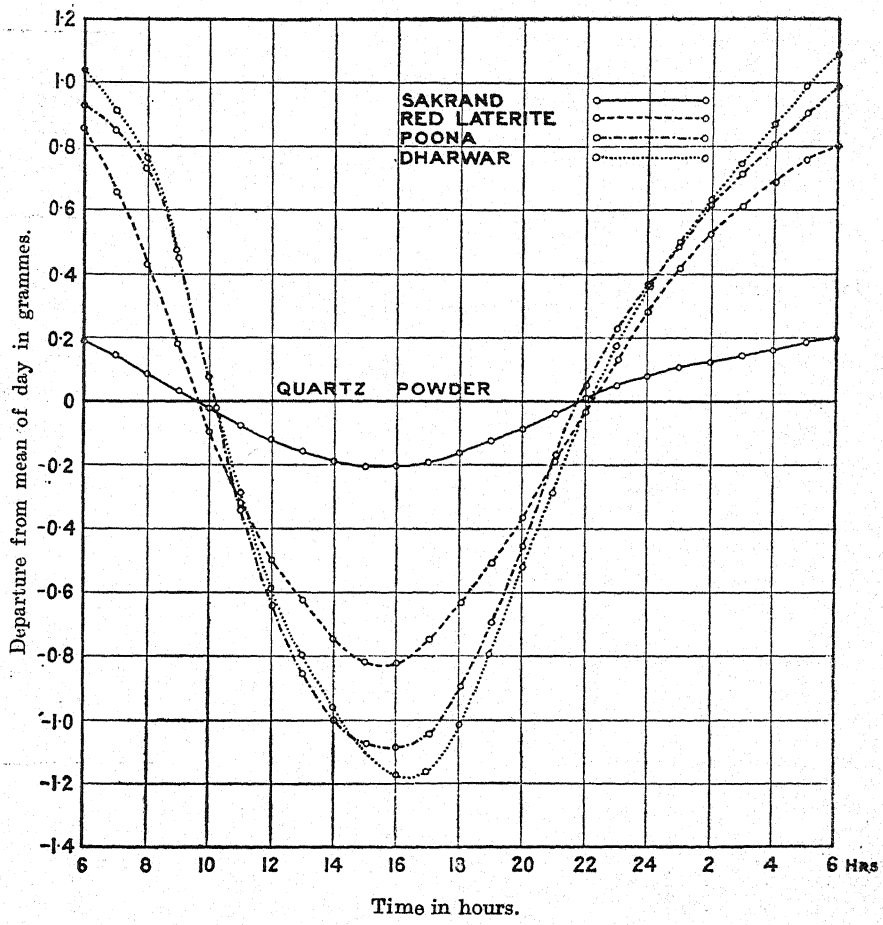


Fig. 2. Hourly variation of moisture-content of five different soils (0600 hours of 1st June 1934 to 0600 hours of 2nd June 1934).

TABLE II

Name of soil	Maximum weight (morning) grms.	Minimum weight (afternoon) grms.	Diurnal range of moisture content grms.
Quartz powder	60·610	60·610	0·000
Sakrand soil	60·575	60·160	0·415
Red laterite	61·080	59·390	1·690
Poona soil (black cotton)	61·400	59·350	2·050
Dharwar (brown schist)	61·450	59·220	2·230

2. Diurnal variation of moisture in eight soils (0600 hrs. of the 22nd to 0600 hrs. of the 23rd January 1935)

It was possible to collect more soil samples from different parts of the country and study the diurnal variations in their moisture contents in January 1935. The experimental procedure was similar to that described in the previous sub-section. Table III gives the particulars regarding the soil samples used.

TABLE III

Name of soil	Colour of the soil	Area of cross-section of vessels used for exposing the samples	Mean weight of soil in grms
Poona	Black	12·6 sq. cm.	30·16
Sholapur	Black	,,	30·46
Hagari	Dark	,,	29·75
Ranchi	Red	,,	29·90
Jhelum	Reddish grey	,,	30·31
Bangalore	Red	,,	30·46
Pusa	Grey (alluvial)	,,	29·75
Lyallpur	Dark-grey	,,	30·33

The hourly weights of these soil samples, when exposed to identical weather conditions from 0600 hrs. of the 22nd to 0600 hrs. of the 23rd January 1935, as well as their departures from the respective mean weights are given in Table IV. Fig. 3 which shows these results diagrammatically confirms the features shown by Fig. 2.

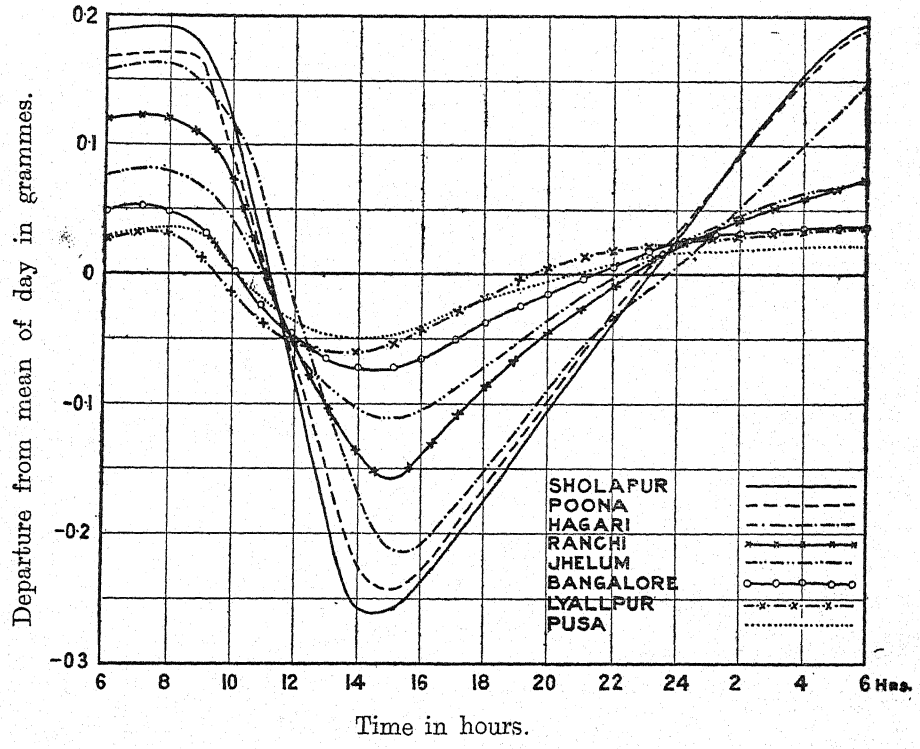


Fig. 3. Hourly variation of moisture-content of eight different soils.

On this day there were traces of clouds in the afternoon which decreased rapidly towards the evening, the night being practically clear. The hourly march of soil temperature, air temperature, humidity percentage, pressure of water vapour, wind and cloudiness are shown in Fig. 4. Apart from the slight cloudiness in the afternoon the day was one of the winter type without any sea-breeze. The stratification of the air layers at night (coldest air being a few inches above the ground and then temperature increasing with height) was undisturbed. The reversal of the vapour pressure gradient may be seen in Fig. 4. The nocturnal desiccating influence of the soil on the air layers near it is also clearly seen.

Temperature in °C. of soil surface and of air at 0·3″ and 1′ above surface.

Humidity percentage at 0·3″ above ground.

Vapour pressure (mm. of Hg.) near surface of ground and at 6 ft. above ground.

Wind velocity m.p.h.

Duration of clear skies. If sunshine recorder shows clear skies during the whole of an hour the value is 1·0, if cloudy the value is 0·0.

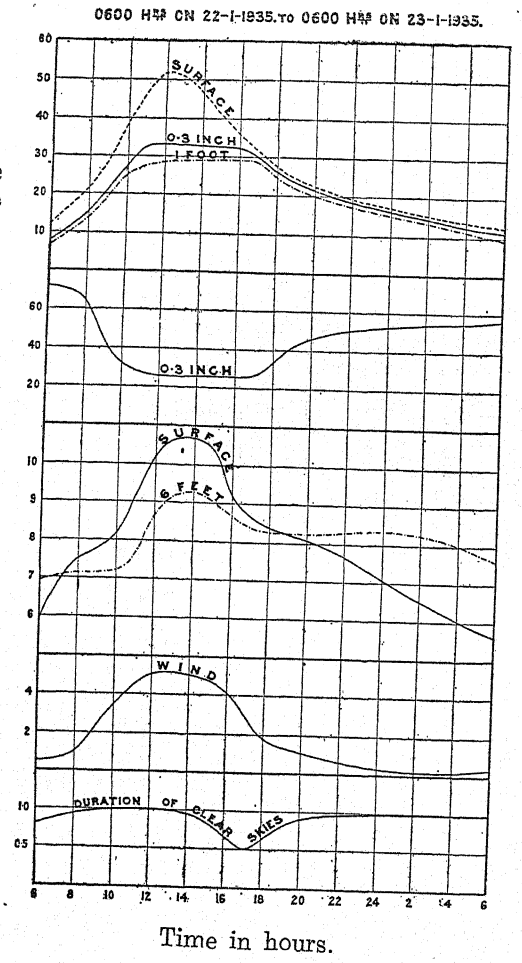


Fig. 4. Hourly variation of meteorological factors (0600 hours of 22nd January 1935 to 0600 hours of 23rd January 1935).

TABLE IV

Time in hours (I. S. T.)	Poona soil		Sholapur soil		Hagari soil		Ranchi soil (Plot 4)		Jhelum soil (305/1930)		Bangalore soil		Pusa soil		Lyallpur soil	
	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day
0600	54·705	0·161	59·115	0·186	60·395	0·156	56·176	0·119	56·745	0·076	64·660	0·049	68·443	0·028	58·876	0·027
0700	54·710	0·166	59·120	0·191	60·400	0·161	56·180	0·123	56·750	0·081	64·665	0·054	68·446	0·031	58·880	0·031
0800	54·719	0·175	59·120	0·191	60·400	0·161	56·177	0·120	56·750	0·081	64·660	0·049	68·455	0·040	58·880	0·031
0900	54·710	0·166	59·105	0·176	60·385	0·146	56·165	0·108	56·738	0·069	64·648	0·037	68·451	0·036	58·862	0·013
1000	54·635	0·091	59·045	0·116	60·355	0·116	56·133	0·076	56·714	0·045	64·615	0·004	68·416	0·001	58·835	—0·014
1100	54·560	0·016	58·955	0·026	60·290	0·051	56·060	0·003	56·666	—0·003	64·585	—0·026	68·395	—0·020	58·805	—0·044
1200	54·482	—0·062	58·850	—0·079	60·214	—0·025	56·000	—0·057	56·612	—0·057	64·564	—0·047	68·380	—0·035	58·793	—0·056
1300	54·395	—0·149	58·745	—0·184	60·145	—0·094	55·961	—0·096	56·585	—0·084	64·546	—0·065	68·368	—0·047	58·790	—0·059
1400	54·320	—0·224	58·670	—0·259	60·080	—0·159	55·920	—0·137	56·564	—0·105	64·540	—0·071	68·368	—0·047	58·790	—0·059
1500	54·300	—0·244	58·670	—0·259	60·030	—0·209	55·900	—0·157	56·558	—0·111	64·540	—0·071	68·368	—0·047	58·799	—0·050
1600	54·314	—0·230	58·700	—0·229	60·033	—0·206	55·920	—0·137	56·567	—0·102	64·546	—0·065	68·377	—0·038	58·811	—0·038
1700	54·347	—0·197	58·726	—0·203	60·061	—0·178	55·955	—0·102	56·582	—0·087	64·561	—0·050	68·386	—0·029	58·820	—0·029
1800	54·380	—0·164	58·756	—0·173	60·090	—0·149	55·970	—0·087	56·600	—0·069	64·579	—0·032	68·395	—0·020	58·829	—0·020
1900	54·416	—0·128	58·790	—0·139	60·120	—0·119	55·992	—0·065	56·615	—0·054	64·591	—0·020	68·402	—0·013	58·844	—0·005
2000	54·455	—0·089	58·826	—0·103	60·154	—0·085	56·012	—0·045	56·636	—0·033	64·603	—0·008	68·410	—0·005	58·856	—0·007

TABLE IV—*contd.*

Poona soil			Sholapur soil		Hagari soil		Ranchi soil (Plot 4)		Jhelum soil (305/1930)		Bangalore soil		Pusa soil		Lyallpur soil	
Time in hours (I. S. T.)	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day	Weight in grms.	Departure from mean of day
2100	54·500	—0·044	58·868	—0·061	60·187	—0·052	56·030	—0·027	56·654	—0·015	64·615	0·004	68·415	0·000	58·862	0·013
2200	54·525	—0·019	58·904	—0·025	60·202	—0·037	56·048	—0·009	56·666	—0·003	64·620	0·009	68·428	0·013	58·868	0·019
2300	54·551	0·007	58·934	0·005	60·220	—0·019	56·066	0·009	56·681	0·012	64·630	0·019	68·428	0·013	58·871	0·022
2400	54·575	0·031	58·964	0·035	60·250	0·011	56·078	0·021	56·693	0·024	64·640	0·029	68·431	0·016	58·871	0·022
0100	54·600	0·056	58·988	0·059	60·268	0·029	56·090	0·033	56·705	0·036	64·640	0·029	68·434	0·019	58·877	0·028
0200	54·626	0·082	59·021	0·092	60·295	0·056	56·099	0·042	56·717	0·048	64·642	0·031	68·434	0·019	58·880	0·031
0300	54·655	0·111	59·054	0·125	60·316	0·077	56·108	0·051	56·726	0·057	64·642	0·031	68·435	0·020	58·884	0·035
0400	54·685	0·141	59·084	0·155	60·340	0·101	56·120	0·063	56·732	0·063	64·648	0·037	68·436	0·021	58·886	0·037
0500	54·710	0·166	59·102	0·173	60·364	0·125	56·126	0·069	56·738	0·069	64·649	0·038	68·438	0·023	58·886	0·037
0600	54·722	0·178	59·120	0·191	60·385	0·146	56·138	0·081	56·742	0·073	64·649	0·038	68·440	0·025	58·886	0·037
Mean of day	54·544		58·929		60·239		56·057		56·669		64·611		68·415		58·849	

We may now combine the results of Tables I and IV and calculate the moisture variation index of different types of soils (diurnal range of moisture content expressed as a ratio of the diurnal range of the Poona soil). The values of this coefficient are given in Table V for all the soils which have been experimented with.

TABLE V

Name of soil	Moisture variation index (Poona=1)
Poona black cotton soil	1·00
Dharwar black cotton soil	1·10
Sholapur black soil	1·04
Hagari	0·86
Red laterite	0·81
Ranchi	0·65
Jhelum	0·44
Bangalore	0·29
Pusa (alluvial)	0·20
Lyallpur (alluvial)	0·21
Sakrand (alluvial)	0·19
Quartz powder	0·00

The above table shows that the black cotton soils undergo the maximum diurnal variation of moisture content owing to the loss of water by evaporation during the day and gain by absorption from the air during the night. The brown and red soils come next. The alluvial soils from the Indo-Gangetic as well as the Sind areas show a diurnal variation which is only a fifth of that of the black cotton soils. The sample of quartz powder comes at the bottom of the list with no variation. It is indeed remarkable that soils from places at large distances apart show very similar features so long as they belong to the same soil group [Ramdas and Katti, 1935,[1]]. We shall discuss this point at greater length in a later section.

3. Diurnal range of the moisture content of Poona soil as estimated from actual samples taken daily from the soil surface during the period 22nd January 1935 to 5th March 1935

The hourly variation of the moisture content of soil samples discussed in the two previous sub-sections clearly shows that the epochs of maximum and minimum water contents coincide with the epochs of minimum and maximum air temperature respectively. In Table VIII of paper I we have indicated the results of earlier estimations of the moisture content of the soil samples taken from the surface at sunrise and at sunset respectively. In view of the more precise information regarding the epochs of maximum and minimum moisture contents of the soil (which shows that the time of minimum moisture content is much earlier than sunset time) available from the investigations described above, it was considered

worth whileto make daily estimations of the moisture contents of soil samples taken from the surface of the bare soil of the Agricultural Meteorological Observatory at 0630 hrs. and 1530 hrs. I. S. T. respectively in order to find out how the diurnal range of the moisture-content varied from day to day. Such daily estimations were made from the 22nd January to the 5th March 1935. The results are given in Table VI. The moisture-contents are here expressed as percentages of the weight of the dry soil. The differences between these percentages are quite large and systematic. The average moisture content in the morning during the whole period is 7·79 per cent whereas that in the afternoon is 3·80 per cent and the difference of nearly four per cent shows how large is the diurnal variation. This diurnal variation is in agreement with the value obtained by experiments with the soil inside a vessel (Tables II and IV), thus showing that the results of all these experiments support each other satisfactorily.

TABLE VI

Moisture percentage in samples from surface soil taken in the morning and evening at about 6-30 A.M. and 3-30 P.M. respectively

Date	A.M.	P.M.	Date	A.M.	P.M.
22nd Jan. 1935	9·81	5·20	12th Feb. 1935	6·95	3·40
23rd „ 1935	9·12	5·07	13th „ 1935	7·12	3·55
24th „ 1935	8·78	3·82	14th „ 1935	7·83	3·20
25th „ 1935	9·54	5·48	15th „ 1935	7·04	3·06
26th „ 1935	9·23	4·91	16th „ 1935	6·81	3·22
27th „ 1935	8·94	5·06	17th „ 1935	6·78	2·93
28th „ 1935	9·42	5·23	18th „ 1935	6·91	3·30
29th „ 1935	9·45	5·84	19th „ 1935	7·35	3·31
30th „ 1935	8·53	4·21	20th „ 1935	7·22	2·81
31st „ 1935	8·84	5·32	21st „ 1935	6·81	3·64
1st Feb. 1935	9·01	4·92	22nd „ 1935	7·00	3·25
2nd „ 1935	8·62	4·00	23rd „ 1935	6·92	2·89
3rd „ 1935	8·53	4·12	24th „ 1935	6·65	2·54
4th „ 1935	8·04	3·55	25th „ 1935	7·21	3·12
5th „ 1935	7·85	4·05	26th „ 1935	7·13	3·32
6th „ 1935	8·13	4·93	27th „ 1935	7·55	3·34
7th „ 1935	7·32	3·94	28th „ 1935	7·08	3·26
8th „ 1935	7·52	3·41	1st Mar. 1935	6·98	3·15
9th „ 1935	6·84	3·23	2nd „ 1935	7·35	2·94
10th „ 1935	7·21	3·30	3rd „ 1935	7·21	3·30
11th „ 1935	7·32	3·89	4th „ 1935	7·43	3·12
			5th „ 1935	7·51	3·43

Fig. 5 gives the hourly values of the moisture content of the surface soil as estimated on 22nd January 1935. The similarity of the hourly march as shown by this figure to that seen in Figures 1, 2 and 3 is quite striking.

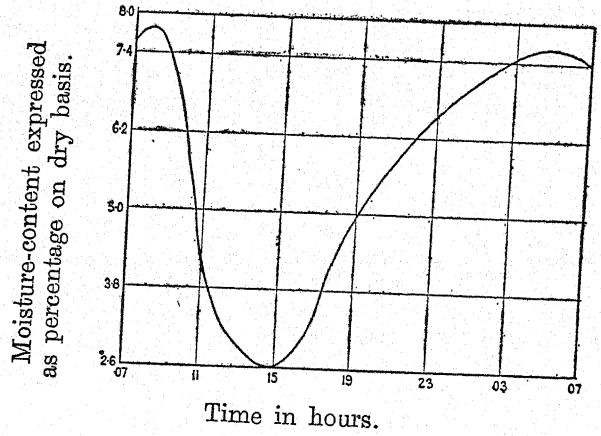


Fig. 5. Hourly variation in the moisture-content of the surface soil (in the open field) on 22nd January 1935.

4. Diurnal variation in the moisture-content of Poona soil in relation to its depth below the soil surface

We have established the following facts in the previous sub-sections : —

(1) there is a conspicuous exchange of moisture between the soil surface and the air layers above it,

(2) the intensity of this exchange is a characteristic property of each soil, and

(3) the epochs of maximum and minimum moisture contents of the soil coincide with those of minimum and maximum temperatures respectively.

In the experiments with samples kept in vessels the soil and the air layers form the two components in the system. Under actual field conditions, however, we have to investigate whether the layers below the surface play any important part in the moisture balance at the surface during clear weather. The problem is much simplified by the fact that, in the Bombay-Deccan, there is no water table which would affect the soil layers near the surface, because the surface layers are resting above hard rock or *murram* and the water table is several feet below. The question whether the soil immediately below the surface makes any measurable contribution of moisture to the surface during the day can be settled easily by taking samples from different depths at intervals during the day and estimating their moisture contents. If there is a significant diurnal variation of the moisture contents at these depths, then it will be necessary to conclude that these layers do take part in the moisture balance at the surface.

TABLE VII

Diurnal variation of soil-moisture (per cent on dry basis) at different depths and of contemporary meteorological factors from 0600 hrs. of 19th February 1935 to 0600 hrs. of 20th February 1935

Time	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700
Percentage moisture at surface	7·0	7·2	6·8	5·9	5·1	4·3	3·7	3·1	2·6	2·7	2·9	3·2
Percentage moisture at 1″ depth	7·6	7·5	7·7	7·5	8·0	8·2	8·3	8·4	8·2	7·9	7·7	7·7
Percentage moisture at 6″ depth	25·7	25·6	25·7	25·0	25·2	25·5	25·0	25·4	25·7	25·8	25·8	25·7
Vapour pressure (0·3″) mm. of Hg.	7·4	7·5	7·9	9·7	11·0	9·0	7·5	6·5	5·8	4·5	2·7	2·7
Vapour pressure (6 ft.) mm. of Hg.	7·8	7·7	7·6	7·4	6·9	5·5	5·0	5·3	5·5	3·8	3·3	3·3
Humidity per cent (0·3″)	65	65	63	52	38	26	20	16	13	10	6	5
Humidity per cent (6 ft.)	66	64	60	52	37	25	19	18	15	12	10	10
Wind speed (in miles per hour)	0·5	0·6	1·0	1·2	1·5	3·6	4·7	4·7	4·4	3·6	2·6	2·1

Time	1800	1900	2000	2100	2200	2300	2400	0100	0200	0300	0400	0500	0600
Percentage moisture at surface	3·5	3·8	4·2	4·6	4·8	4·9	5·1	5·2	5·3	5·3	5·4	5·6	5·9
Percentage moisture at 1″ depth	8·3	8·0	8·0	7·9	7·6	7·3	7·4	7·5	7·7	7·6	7·8	8·0	7·9
Percentage moisture at 6″ depth	25·6	25·3	24·9	24·8	25·5	25·8	25·9	25·9	25·9	25·7	25·5	25·2	25·0
Vapour pressure (0·3″) mm. of Hg.	3·4	3·3	4·0	3·9	4·0	4·1	3·9	3·8	3·5	3·3	3·1	2·8	2·9
Vapour pressure (6 ft.) mm. of Hg.	3·7	3·7	4·0	4·3	4·7	5·1	5·0	4·9	4·8	4·6	4·5	4·4	4·2
Humidity per cent (0·3″)	9	15	19	22	26	29	31	33	34	34	35	35	36
Humidity per cent (6 ft.)	12	15	20	24	32	36	39	42	44	46	47	48	48
Wind speed (in miles per hour)	1·3	1·0	0·8	0·6	0·4	0·3	0·2	0·1	0·1	0·2	0·1	0·1	0·1

TABLE VIII

Diurnal variation of soil-moisture (percentage on dry basis) at different depths and of contemporary meteorological factors from 0600 hrs. of 6th May 1935 to 0600 hrs. of 7th May 1935.

Time	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700
Percentage moisture at surface	6·8	6·6	6·4	5·7	5·3	4·7	4·3	4·0	3·7	3·6	3·9	4·2
Percentage moisture at 1″ depth	7·8	7·6	7·6	7·8	7·9	8·0	8·0	7·8	7·7	7·4	7·3	7·0
Percentage moisture at 6″ depth	20·2	20·4	20·7	20·6	20·6	20·5	20·4	20·4	20·6	20·7	21·0	20·3
Vapour pressure (0·3″) mm. of Hg.	9·5	10·4	11·5	11·0	9·7	8·3	8·0	7·8	7·2	4·5	4·5	8·0
Vapour pressure (6 ft.) mm. of Hg.	10·9	10·5	9·9	9·0	7·7	6·6	6·0	5·4	4·9	4·4	5·0	8·1
Humidity per cent (0·3″)	53	47	34	24	17	11	9	9	7	4	5	12
Humidity per cent (6 ft.)	56	51	35	24	17	13	11	9	8	7	9	16
Wind speed (in miles per hour)	0·5	0·5	0·8	1·5	2·7	3·5	4·2	4·6	5·1	5·3	4·2	5·0

Time	1800	1900	2000	2100	2200	2300	2400	0100	0200	0300	0400	0500	0600
Percentage moisture at surface	4·5	5·0	5·5	5·8	6·1	6·3	6·6	6·8	7·0	7·2	7·3	7·5	7·5
Percentage moisture at 1″ depth	6·8	6·8	6·8	7·0	7·3	7·2	7·4	7·3	7·3	7·5	7·1	7·4	7·6
Percentage moisture at 6″ depth	20·0	20·3	20·6	20·9	21·0	20·9	20·9	20·8	20·7	20·7	20·6	20·6	20·5
Vapour pressure (0·3″) mm. of Hg.	14·5	15·7	16·4	16·7	16·9	16·7	16·4	15·8	15·4	14·8	14·4	13·7	13·5
Vapour pressure (6 ft.) mm. of Hg.	14·1	17·5	18·5	18·8	18·1	17·2	16·7	16·4	16·0	15·6	15·2	14·9	14·6
Humidity per cent (0·3″)	25	37	46	51	53	54	55	56	56	57	57	57	55
Humidity per cent (6 ft.)	27	40	47	56	56	56	57	57	58	58	58	59	59
Wind speed (in miles per hour)	7·7	8·5	8·5	7·5	6·2	5·6	5·1	4·6	4·2	3·8	3·4	2·8	2·4

Experiments to verify this point were made from 19th to 20th February and 6th to 7th May 1935. Soil samples (in duplicate) were taken simultaneously from the surface, one inch and 6 in. below the surface at two-hourly intervals and their moisture-contents determined in the usual manner. Measurements of the meteorological factors were also made simultaneously, and are given in Tables VII and VIII respectively. The moisture-contents of the soil at the three depths and for different hours on these two occasions are shown in Figs. 6 and 7 respectively. The curves in these figures clearly show that the diurnal variation is insignificant except at the surface.

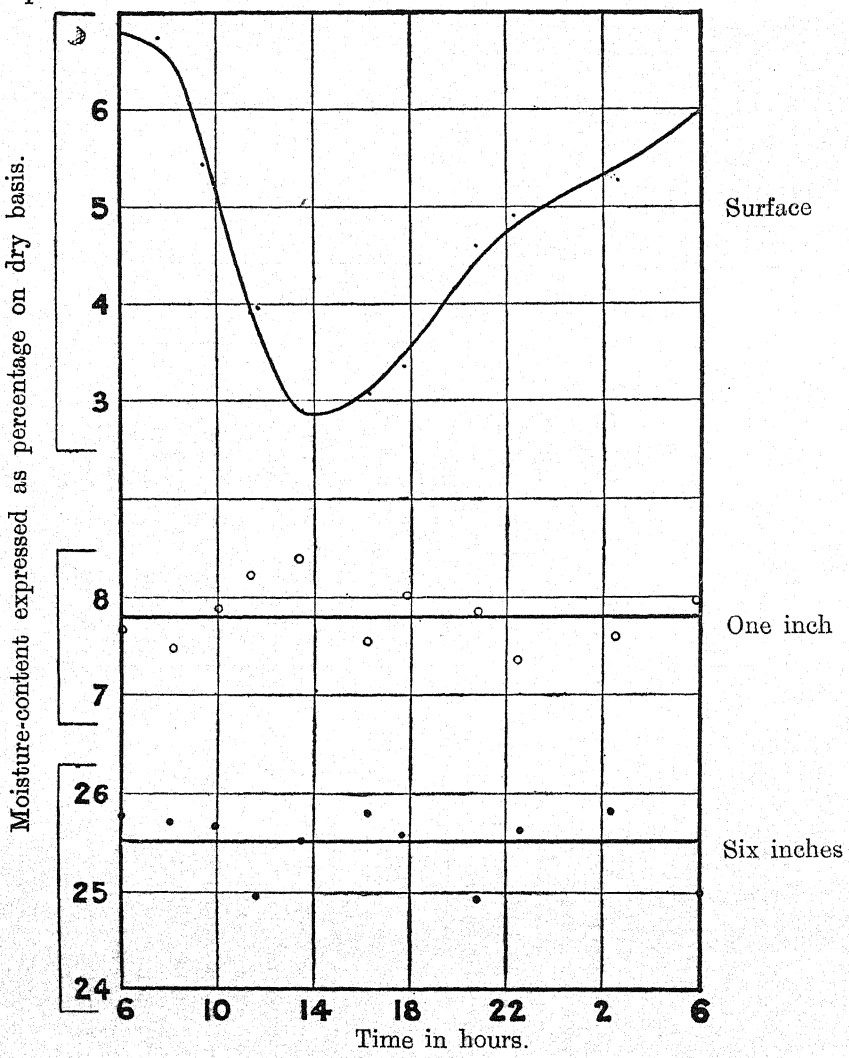


Fig. 6. Hourly variation of moisture-content at "surface", 1" and 6" depths (0600 hours of 19th February 1935 to 0600 hours of 20th February 1935).

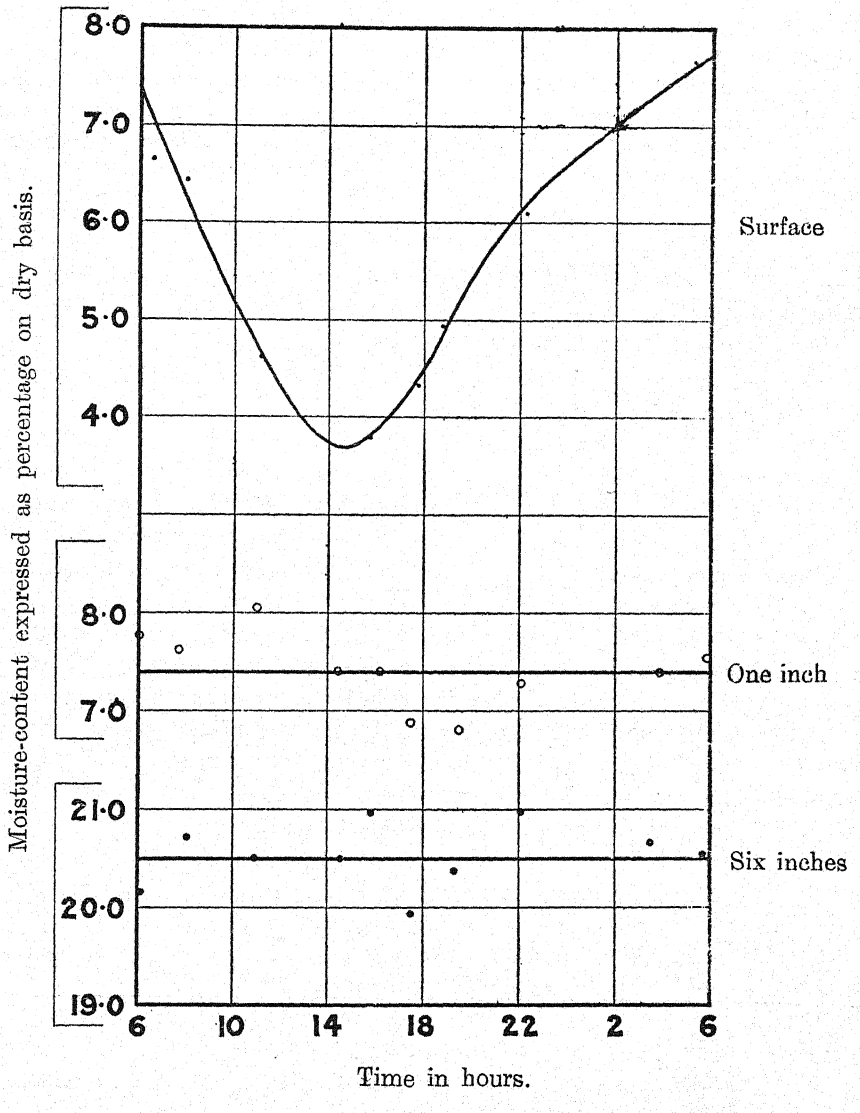


Fig. 7. Hourly variation of moisture-content at "surface", 1", 6" depths (0600 hours of 6th May 1935 to 0600 hours of 7th May 1935).

The soil layers below an inch may, therefore, be assumed to have no influence on the hourly variation of moisture at the surface during the clear season. We are thus concerned in the present discussion mainly with the soil surface and the air layers above it.

It is, of course, to be expected that, in tracts where the water table is very near the surface, *e.g.*, coastal tracts of India and tracts near irrigation canals as in the Punjab and in Sind, the water in the soil below the surface would play a most important role in the moisture balance at the surface of the soil. Phenomena of this type would be quite distinct from what we have been discussing in this paper and deserve a special study.

Section II

The moisture-variation index in relation to other properties of the soil

The results of chemical and mechanical analyses of the main soil types in India are available in the publications of the various agricultural departments. The data are by no means exhaustive or derived according to a common plan or method. Nevertheless, an examination of these data reveals that one of the major variations from soil to soil is in the clay fraction. Table IX gives the results of mechanical analyses of certain typical soils. The table shows that, in general, the alluvial group has a small clay fraction and large silt content, the black soils are rich in clay and poorer in the particles of large size, other soils having intermediate positions in the scale of clay content. It is well known that, the larger the clay fraction or colloidal matter in a medium like the soil, the larger will be the effective area available for absorptive phenomena, so that, in a general way, it is easy to see why black cotton soils should have the largest moisture-variation index.* The study of the moisture-variation index in the case of the different groups of particle sizes would certainly be worth-while in the case of the major soils of India as a special investigation. In the present section we shall confine ourselves to the results of some preliminary experiments on the physical properties of the soils which were made recently at Poona.

TABLE IX

Name of soil types	District	Clay	Fine silt	Silt	Fine sand	Coarse sand	Remarks
1. Black cotton soils	South Gujarat	44·4	8·1	26·5	20·7	0·4	
	Jalgaon	49·1	4·0	17·1	25·6	4·1	Taken from bulletin No. 160, 1929 "Soils of the Bombay Presidency."
	Surat	32·3	6·4	34·8	26·3	0·2	
	West Khandesh	42·8	8·7	32·1	14·1	4·1	
	Poona	25·7	8·1	33·3	25·9	7·0	
	Nagpur	44·4	18·3	12·8	5·5	0·4	Data supplied by Agricultural Department, Central Provinces.
	Akola	36·9	19·1	15·8	8·5	2·6	
	Wardha	40·0	21·7	11·2	7·3	0·4	

* "A Study of Absorption of Moisture by Soil" by J. N. Sen, Ph. D., and B. M. Amin, B.A., Memoirs of the Department of Agriculture in India (Chemical Series), Vol. 8, No, 12, 1926, is very interesting in this connection.

TABLE IX—(*contd.*)

Name of soil types	District	Clay	Fine silt	Silt	Fine sand	Coarse sand	Remarks
2. Laterite and red soils	Poona (from hill sides).	4·0	3·9	19·9	18·3	13·3	From bulletin No. 160, Department of Agriculture, Bombay Presidency.
	Dacca .	28·1	13·5	24·5	21·2	4·0	Data supplied by Agricultural Department, Bengal.
	Mandalay	10·0 22·3 10·8	21·1 16·0 21·0	23·2 23·8 25·9	39·3 33·6 33·1	5·5 4·0 9·2	Taken from Agricultural Survey No. 15 of 1932, Department of Agriculture, Burma.
3. Alluvial soils	Sind .	15·7	8·5	56·1	19·5	0·2	From bulletin No. 160, Department of Agriculture, Bombay Presidency.
	Punjab (average of typical districts).	12·3	21·2	20·7	30·0	12·0	From Memoirs of the Department of Agriculture in India, " Soils of the Punjab ", Vol. X, No. 2.
	Bengal .	14·1	20·5	26·1	27·8	1·24	Data supplied by Department of Agriculture, Bengal.

1. Water-holding capacity

The water-holding capacities of soils from Poona, Sholapur, Hagari, Bangalore, Pusa and Lyallpur were determined with the help of portable drain-gauges designed locally. Plate LXVI is a photograph of this type of drain-gauge which consists of a cylindrical vessel of the required height and 5 inches in diameter (same as that of the standard rain-gauge) to contain the soil and a funnel which drains the water percolating through the soil into a suitable receiver below. The soil rests on a perforated disc at the bottom of the cylindrical vessel.

In our experiments 6″ of each soil were packed uniformly at the mean rate of 17½ oz. per inch of depth. After all the gauges were filled with the different soils, one inch depth of water was poured at the top of the soil at 1400 hrs. daily and any percolated water measured on the succeeding day at the same time. These experiments were continued without break until the soils were saturated with water when they began to drain all the water poured at the top of the soil columns.

The cylindrical vessels of the drain gauges were kept covered carefully to prevent loss of water by evaporation, the covers being removed only momentarily daily once for pouring water.

Table X gives the results of one of these experiments. After pouring one inch of water on the 4th and 5th December 1934, no percolation was noticed through any of the soils. After pouring one inch on the 6th, the Lyallpur soil showed on the 7th at 1400 hours a percolation of 0·88 in., Bangalore 0·69 in., Pusa 0·62

in., and Hagari 0·22 in. The Sholapur and Poona soils still showed no percolation. The addition of one inch on the 7th started percolation in these two also so that, after the 8th, the soils all began to percolate all the water poured at the top. From these results simple subtraction of the total percolation from the total water added during the period of the experiment gives the quantity of water "held" by the soil in each case. The water-holding capacities so measured are expressed as percentage of air-dry soil in Table XI. Here also the black cotton soils top the list.

TABLE X

Date	Depth of water poured into each gauge at 1400 hrs.	Amount of water which had percolated through the soil during 24 hours ending 1400 hours of the next day					
		Sholapur	Poona	Hagari	Pusa	Bangalore	Lyallpur
4th December 1934 . .	One inch	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
5th December 1934 . .	,,	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
6th December 1934 . .	,,	*Nil*	*Nil*	0·22″	0·62″	0·69″	0·88″
7th December 1934 . .	,,	0·31″	0·38″	0·99″	0·99″	0·96″	0·96″
8th December 1934 . .	,,	1·00″	0·97″	0·99″	0·99″	0·97″	0·96″

TABLE XI

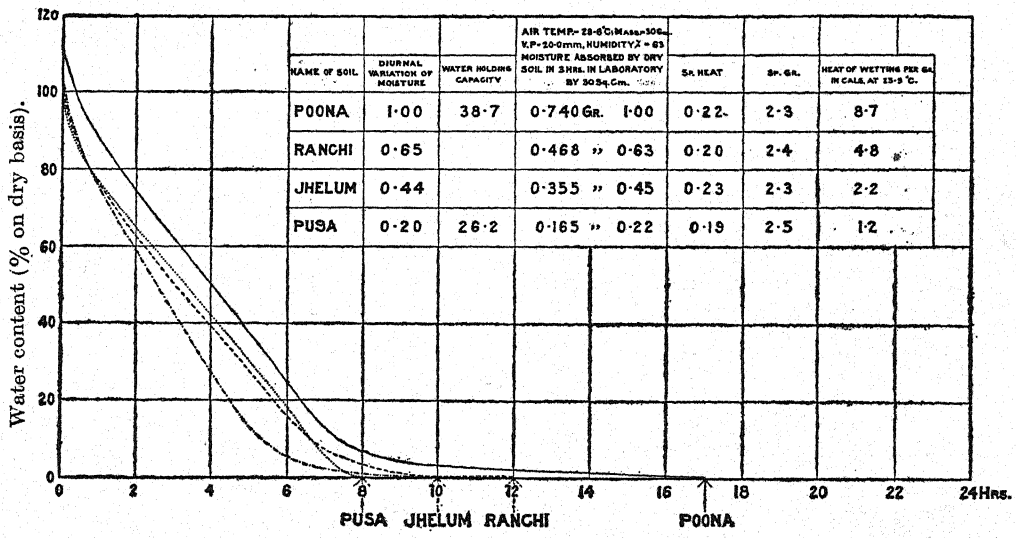
Name of soil	Depth Inches	Amount of soil per inch of depth Oz.	Total amount of water (in inches) required for saturation Inches	Water-holding capacity expressed as percentage on air-dry basis
Sholapur . .	6	17·5	3·69	41·0
Poona . . .	6	17·5	3·65	38·7
Hagari . . .	6	17·5	2·80	30·9
Pusa . . .	6	17·5	2·40	26·2
Bangalore . .	6	17·5	2·38	24·0
Lyallpur . .	6	17·5	2·20	22·8

2. Rate of drying

For determining the moisture-content of a soil sample, the sample is first weighed and kept in a steam oven until it has lost all the moisture it can give up at 100°C. This is usually verified by weighing until successive weights are

constant. An exact study of the rate of drying in a steam oven should be interesting both from a practical point of view as well as from the point of view of this paper.

Four typical soils, *viz.*, Poona, Ranchi, Jhelum and Pusa soils, were chosen for such a study ; 30 grms. of each of these soils were mixed with about the same weight of water and kept in the steam oven at the same time after weighing initially. The vessels were weighed at hourly intervals, care being taken to keep them air-tight while weighing. The hourly weighings were discontinued in each case after the particular soil had attained a constant minimum weight at 100°C. The rate of drying as well as the total time taken by each of the soils for complete drying at 100°C. will be seen from the curves in Fig. 8. The curves show that, when the soils have about 100 per cent water on dry basis, the rate of drying is rapid until the water content goes down to about 90 per cent. Later, the soils go on drying at a small but uniform rate until the moisture-content decreases to about 5 per cent. Further decrease in the moisture-content takes the least time in the case of the Pusa soil and most time in the case of Poona soil, the other soils taking an intermediate position between these.



NAME OF SOIL	DIURNAL VARIATION OF MOISTURE	WATER HOLDING CAPACITY	AIR TEMP.-28·8°C.M.Asp.-30%, V.P.-20·0mm, HUMIDITY% = 63 MOISTURE ABSORBED BY DRY SOIL IN 3 HRS. IN LABORATORY BY 30·54 Cm.	SP. HEAT	SP. GR.	HEAT OF WETTING PER GM. IN CALS. AT 23·5 °C.
POONA	1·00	38·7	0·740 GR. 1·00	0·22	2·3	8·7
RANCHI	0·65		0·468 " 0·63	0·20	2·4	4·8
JHELUM	0·44		0·355 " 0·45	0·23	2·3	2·2
PUSA	0·20	26·2	0·165 " 0·22	0·19	2·5	1·2

Fig. 8. The curves in the figures show the rates of drying of four different types of soils at 100°C. The inset table gives :—

(*a*) the diurnal variation of moisture-content (expressed in terms of Poona =1),

(*b*) the water-holding capacity,

(*c*) the absorption of moisture in 3 hours under steady conditions in the laboratory,

(*d*) the specific heat,

(*e*) the specific gravity, and

(*f*) the heat of wetting of the above soils.

Water content of soil (% on dry basis).

Further experiments indicated that the initially rapid drying is due to evaporation as from a free water surface at 100°C., the later steady but less rapid drying is due to evaporation of the capillary water from the moist soil and the last stage (after about the 5 per cent stage) is due to evaporation of the hygroscopic water. Pusa soil takes eight hours, Jhelum soil ten hours, Ranchi soil twelve hours and Poona soil seventeen hours to attain a steady minimum weight after complete drying at 100 °C. These times are in the same order as the moisture-variation indices given in the inset table of Fig. 8.

3. Rates of absorption of moisture under steady conditions in the laboratory

The experiments described in the first section of this paper were made in the open with soils exposed to the diurnal variations of the various meteorological factors. In order to verify whether the soils would show the same sequence in their power to absorb moisture under the steady conditions inside the laboratory, as they do with respect to their moisture-variation indices in the open, experiments were made with four typical soils, *e.g.*, Poona, Ranchi, Jhelum and Pusa soils. About thirty gr. of each of these soils which had been previously dried completely at 100°C., were exposed after they had attained room temperature to the still air of the laboratory in vessels having an area of cross-section of 30 sq. cms., at room temperature. The air temperature was 29·8°C., the partial pressure of water vapour 20·0 mm. and the percentage humidity 63 per cent. Under these conditions the Poona soil absorbed 0·740 gr. of moisture, the Ranchi soil absorbed 0·468 gr. of moisture, the Jhelum soil absorbed 0·355 gr. of moisture and the Pusa soil 0·165 gr. of moisture in three hours. Expressed in terms of Poona soil equal to 1, these absorption are in the ratio of 1 : 0·63 : 0·45 : 0·22, which is practically identical with the ratio of the moisture-variation indices as given in Table V.

4. Specific gravity

The true specific gravity of soils from Poona, Ranchi, Jhelum and Pusa was determined by the specific gravity bottle method, the liquid used being kerosene. The specific gravity of kerosene was also determined for use in the calculations. The specific gravity of these soils varies between 2·3 and 2·5.

TABLE XII

Name of soil	Specific gravity
Poona soil	2·27
Ranchi soil	2·40
Jhelum soil	2·35
Pusa soil	2·51

5. Specific heat

The specific heats of the same soils were determined by pouring hot water into a calorimeter containing a suspension of about forty grms. of soil in water

(known quantities of soil and water are mixed prior to the experiment and allowed to stand until the heat of wetting is dissipated and room temperature is attained by the mixture). The increase of temperature of the mixture was noted and the specific heat of the soil calculated in the usual manner. The table below gives the values of specific heats of the different soils. We shall have to use these values later in calculating the heat of wetting of the soils (next section).

TABLE XIII

Name of soil	Specific heat
Poona soil	0·22
Ranchi soil	0·20
Jhelum soil	0·23
Pusa soil	0·19

6. Heat of wetting

The heat of wetting with water was determined for soils from Poona, Ranchi, Jhelum and Pusa. Samples of these were heated to dryness at 100°C. and then allowed to cool in air-tight vessels. About thirty grm. of each soil* and water both initially at room temperature (25·9°C.) were mixed quickly in a small calorimeter kept inside a Dewar's flask, and the rise of temperature noted with a sensitive thermometer. The specific heat of the soil having been measured already, and the water equivalent of the calorimeter determined to start with, the heat of wetting per grm. of dry soil was calculated. The estimated heats of wetting are given below :—

TABLE XIV

Name of soil	Heat of wetting per gr. of soil in grm. calories (at 25·9° C.)
Poona	8·7
Ranchi	4·8
Jhelum	2·2
Pusa	1·2

To summarise the results of these experiments [Ramdas and Katti, 1935, 2] it is seen that (excepting of course the specific heat and true specific gravity) the following properties of soils vary in a similar manner (please also see Fig. 8 and the inset table in this connection) :—

(1) Diurnal variation of moisture,
(2) Water-holding capacity,
(3) Absorption of moisture in a definite time interval under steady laboratory conditions, and
(4) Heat of wetting.

* The determinations of absorption, specific gravity, specific heat and heat of wetting were all made with soils which had been passed through a 1 mm. sieve.

Section III

Laboratory determination of the desiccating power of different soils

We have seen that soils, when dry, absorb water vapour from the atmosphere and thus influence the distribution of water vapour in the air layers near the ground. To the meteorologist these results are interesting because the absorption of water vapour by the soil during the night and the evaporation of water from the soil during day time help to explain the observed diurnal variations in the pressure of water vapour. The fact that the diurnal variation of soil-moisture is confined to the first few inches of the soil also explains why a mulch helps to give a blanketing effect to the soil below and to conserve the moisture in the lower layers.*

It would be interesting also to study the efficiency of different soils as desiccating agents under steady conditions in the laboratory. The method adopted was to pass an air current (whose initial humidity was known) above dry soil and to note the decrease of humidity after passage.

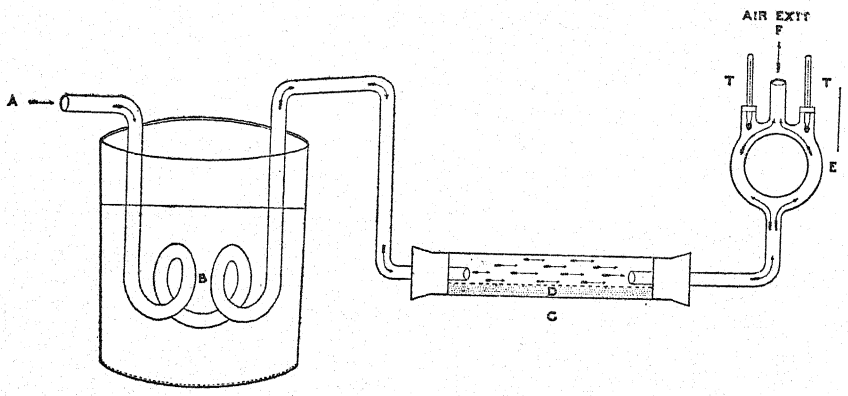


Fig. 9. Apparatus for the measurement of the desiccation of a current of air by different soils.

The experimental arrangement is shown in Fig. 9. The room air compressed by a blower passes into the spiral copper tube B and thence into the tube C which contains the dry soil. The copper spiral B is kept immersed in a water-bath at room temperature so as to maintain the initial conditions constant. This precaution is necessary because the blower gets warmer and warmer as it works and raises the temperature of the air current. After passing through the soil tube

*In this connection please see an interesting article on "The soil as a water reservoir" by T. Eden, *The Tea Quarterly, The Journal of the Tea Research Institute of Ceylon*, Vol. 8, part I, April 1935, p. 31.

C the desiccated air passes into the psychrometer E which has the dry-bulb thermometer T on one side and the wet-bulb thermometer T′ on the other. The thermometers T and T′ are screwed in position and may be removed easily for inspection or for wetting the muslin of the wet bulb thermometer. After passing by the bulbs of T and T′ the air finally emerges at F out into the room. To measure the initial humidity of the air current the tube C is removed and the air passed directly into E. In the initial experiments two psychrometric elements were used, one before and the other after the air current had passed through C, but it was found that the wet bulb of the first would then make the air current entering C more humid than the room air. During the course of the experiment readings of an Assmann Psychrometer were also taken at intervals in the room air outside the apparatus. The tube C was connected only after verifying that the pressure of water vapour in the air current as indicated by E was the same as that indicated by the Assmann Psychrometer. This was achieved by increasing the velocity of the air current in the apparatus until the depression of the wet bulb in E was maximum.

After ensuring that the tube psychrometer would function as efficiently as an Assmann Psychrometer and after recording the initial conditions, the tube C containing a known quantity of soil previously dried at 100°C. and spread uniformly along the tube, was inserted. Almost immediately the wet bulb T′ began to fall and within a few minutes attained a steady low value. The dry bulb T did not undergo any variation whatever as the heat of adsorption in the tube C was dissipated rapidly. The extent of the fall in T′ indicates the desiccating effect of the soil in C. The readings of T and T′ were recorded at short intervals of time until the soil in C was exhausted and T′ began to rise and ultimately attained the initial value again. The maximum fall of T′ as well as the time it took to regain the initial value differed from soil to soil, being large in the case of the black cotton soils and small in the case of soils of the alluvial type.

The velocity of the air current was measured during the experiment by noting the time taken for the displacement of a known volume of water. Also, the weight of soil in the tube C was noted both at the commencement and at the end of each experiment.

These experiments were performed with four soils, *viz.*, soils from Poona, Jalgaon, Bangalore and Lyallpur. For comparing the desiccating power of these soils with that of a standard substance the experiment was also repeated with a sample of " Sorbsil " supplied by the Cambridge Instrument Company. Tables XV to XIX give the full experimental details for the different substances in the order in which they are mentioned above.

TABLE XV

Poona soil

Time in minutes	Temperature and humidity of air current				Temperature and humidity of room air (with Assmann Psychrometer)				Other details
	Dry bulb T °C.	Wet bulb T′ °C.	Humidity percentage	Vapour pressure in mm.	Dry bulb °C.	Wet bulb °C.	Humidity percentage	Vapour pressure in mm.	
0	23·3	14·5	37	8·0	23·8	14·6	36	7·9	
½	23·3	13·8	33	7·1	..	..	..	..	
1	23·3	11·5	21	4·4	..	..	..	..	Weight of dry soil before passing air = 11·380 grms.
1½	23·3	10·2	14	2·9	..	..	..	..	Weight of dry soil after passing air = 12·030 grms.
2	23·3	9·5	10	2·2	..	..	..	..	Weight of water vapour absorbed by the soil = 0·650 grm.
3	23·3	9·0	8	1·6	..	..	..	..	
5	23·3	8·9	7	1·5	24·3	15·0	36	8·1	
8	23·3	8·9	7	1·5	..	..	..	..	
10	23·3	9·0	8	1·6	..	..	..	..	Velocity of air current = 43·8 c.c. per second.
14	23·2	9·3	9	1·8	..	..	..	..	
18	23·3	9·7	10	2·2	..	..	..	..	
24	23·4	10·4	14	3·0	..	..	..	..	
30	23·4	10·9	16	3·5	..	..	..	..	Diameter of tube C = 1·3 cm.
35	23·8	11·4	18	3·9	..	..	..	..	Length of tube C = 1 metre.
40	23·8	11·8	20	4·4	24·5	15·0	35	8·0	Fraction of volume of C actually occupied by the soil = $\frac{1}{10}$
50	24·0	12·0	23	5·2	..	..	..	..	
60	23·9	13·2	27	6·0	..	..	..	..	
70	24·3	13·8	29	6·6	..	..	..	..	
80	24·5	14·2	30	7·0	25·0	14·8	32	7·5	
90	24·8	14·5	31	7·2	25·2	14·4	29	6·9	

TABLE XVI

Jalgaon soil

Time in minutes	Temperature and humidity of air current				Temperature and humidity of room air (with Assmann Psychrometer)				Other details
	Dry bulb T °C.	Wet bulb T′ °C.	Humidity percentage	Vapour pressure in mm.	Dry bulb °C.	Wet bulb °C.	Humidity percentage	Vapour pressure in mm.	
0	25·3	15·1	32	7·8	25·2	15·2	33	8·0	Weight of dry soil before passing air = 11·710 grms.
1	25·3	12·5	18	4·5	..	..	..	..	Weight of dry soil after passing air = 12·280 grms.
1½	25·3	11·2	12	2·9	..	..	..	..	Weight of water vapour absorbed by the soil . = 0·570 grm.
2	25·3	10·7	10	2·3	..	..	..	..	Velocity of air current . . = 43·8 c.c. per second.
3	25·3	10·3	8	1·9	25·4	15·2	32	7·9	Diameter of tube C. = 1·3 cm.
4	25·3	10·2	7	1·8	..	..	..	..	Length of tube C . = 1 metre.
5	25·3	10·2	7	1·8	..	..	..	..	Fraction of volume of C actually occupied by the soil. = $\frac{1}{10}$
6	25·2	10·3	8	1·9	..	..	..	..	
8	25·2	10·5	9	2·2	..	..	..	..	
10	25·2	10·6	10	2·3	..	..	..	..	
20	25·2	11·3	13	3·1	25·6	15·5	33	8·2	
25	25·2	11·6	14	3·4	..	..	..	..	
30	25·4	12·1	16	3·9	..	..	..	..	
35	25·4	12·3	17	4·2	..	..	..	..	
40	25·4	12·9	20	4·9	..	..	..	..	
50	25·8	13·8	23	5·8	..	..	..	..	
60	25·8	14·4	27	6·6	..	..	..	..	
70	25·8	15·0	30	7·4	..	..	..	..	
80	26·2	15·5	31	7·9	26·0	15·4	31	7·8	

TABLE XVII

Bangalore soil

Time in minutes	Temperature and humidity of air current				Temperature and humidity of room air (with Assmann Psychrometer)				Other details
	Dry bulb T °C.	Wet bulb T′ °C.	Humidity percentage	Vapour pressure in mm.	Dry bulb °C.	Wet bulb °C.	Humidity percentage	Vapour pressure in mm.	
0	25·0	14·1	28	6·6	24·8	14·0	29	6·6	Weight of dry soil before passing air = 11·340 grms.
½	25·0	13·2	23	5·5	..	..	..	..	Weight of dry soil after passing air = 11·370 grms.
1	25·0	11·8	16	3·8	..	..	..	..	Weight of water vapour absorbed by the soil . = 0·030 grm.
1½	25·0	11·6	15	3·5	..	..	..	..	
2	24·8	11·4	15	3·5	..	..	..	..	Velocity of air current . . = 43·8 c.c. per second.
3	24·8	11·8	17	3·9	..	..	..	..	
4	24·8	12·3	19	4·5	..	..	..	..	Diameter of tube C = 1·3 cm.
5	24·8	13·0	23	5·3	..	..	..	..	Length of tube C = 1 metre.
6	24·8	13·4	25	6·0	..	..	..	..	Fraction of volume of C actually occupied by the soil. = $\frac{1}{10}$
7	24·8	13·8	27	6·3	..	..	..	..	
8	24·8	14·0	28	6·6	..	..	..	..	
10	24·8	14·2	29	6·8	24·8	14·0	29	6·6	

TABLE XVIII

Lyallpur soil

Time in minutes	Temperature and humidity of air current				Temperature and humidity of room air (with Assmann Psychrometer)				Other details
	Dry bulb T °C.	Wet bulb T' °C.	Humidity percentage	Vapour pressure in mm.	Dry bulb °C.	Wet bulb °C.	Humidity percentage	Vapour pressure in mm.	
0	25·4	14·0	26	6·3	25·5	14·3	27	6·6	Weight of dry soil before passing air = 11·400 grms.
½	25·4	13·0	21	5·0	..	..	..	..	Weight of dry soil after passing air = 11·427 grms.
1	25·4	12·0	16	3·8	..	..	..	..	Weight of water vapour absorbed by the soil . = 0·027 grm.
1½	25·4	11·7	14	3·5	..	..	..	..	
2	25·4	12·0	16	3·8	..	..	..	..	Velocity of air current . . = 43·8 c.c. per second.
3	25·4	12·3	17	4·2	..	..	..	..	
4	25·4	12·8	20	4·8	..	..	..	..	Diameter of tube C = 1·3 cm.
5	25·4	13·3	22	5·4	..	..	..	..	Length of tube C = 1 metre.
6	25·4	13·7	24	5·9	..	..	..	..	Fraction of volume of C actually occupied by the soil. = $\frac{1}{10}$
7	25·4	14·0	26	6·3	..	..	..	..	
8	25·4	14·1	26	6·4	25·4	14·2	27	6·5	

TABLE XIX

Sorbsil

Time	Temperature and humidity of air current				Temperature and humidity of room air				Other details
	Dry bulb °C.	Wet bulb °C.	Humidity percentage	Vapour pressure in mm.	Dry bulb °C.	Wet bulb °C.	Humidity percentage	Vapour pressure in mm.	
0	30·5	17·7	27	8·8	30·5	17·6	26	8·6	
½ min.	30·4	15·5	17	5·7	..	..	..	..	Weight of dry sorbsil before passing air . . =12·000 grms.
1 min.	30·3	14·3	13	4·2	..	..	..	..	
2 min.	30·3	13·5	10	3·2	..	..	..	..	Weight of sorbsil after passing air =13·430 grms.
3 min.	30·3	13·3	9	2·9	..	..	..	..	
5 min.	30·3	13·6	10	3·3	..	..	..	..	Weight of water vapour absorbed by sorbsil . =1·430 grms.
8 min.	30·3	13·7	11	3·5	..	..	..	..	
12 min.	30·4	14·0	12	3·8	..	..	..	..	Velocity of air current . . =43·8 c.c. per second.
20 min.	30·5	14·2	12	4·0	..	..	..	..	
26 min.	30·9	14·5	12	4·2	31·1	17·5	24	8·2	Diameter of tube C =1·3 cm.
30 min.	30·7	14·7	13	4·4	..	..	..	..	Length of tube C =1 metre.
36 min.	30·5	15·0	15	5·0	30·7	17·6	26	8·5	Fraction of volume of C actually occupied by sorbsil $=\frac{4}{25}$
42 min.	30·6	15·1	15	5·1	..	..	..	..	
48 min.	30·8	15·3	16	5·4	31·1	17·5	24	8·2	

1 hour 4 min.	31·3	15·6	16	5·4	..	..	..	..
1 hour 16 min.	30·7	15·8	18	6·0	..	..	..	..
1 hour 33 min.	31·0	16·2	19	6·4	..	..	..	..
1 hour 43 min.	31·0	16·3	19	6·5	..	..	..	..
1 hour 53 min.	31·1	16·5	20	6·8	31·5	17·3	22	7·7
2 hours	31·2	16·7	21	7·0	..	..	..	..
2 hours 10 min.	31·5	16·8	21	7·3	..	..	..	..
2 hours 20 min.	31·2	16·9	21	7·3	31·5	17·0	21	7·3
2 hours 30 min.	31·2	16·9	21	7·3	..	..	..	..
2 hours 40 min.	31·3	16·9	21	7·3	..	..	..	..
2 hours 50 min.	31·3	16·9	21	7·3	31·5	17·0	21	7·3

The velocity of the air current was adjusted so as to allow 43·8 c.c. of air per second to pass through the apparatus. After passage above the soil exposed in the drying tube C, the wet bulb temperature rapidly fell in the first few minutes and slowly rose again. Each experiment was concluded as soon as the air current ceased to be desiccated, *i.e.*, as soon as the soil had got exhausted and no longer absorbed any moisture from the air current. When this stage was reached the humidity of the air current was practically identical with that of the room air outside the apparatus.

In the case of Poona and Jalgaon soils and of sorbsil, the time taken for completely exhausting the desiccating power was of the order of 80 to 150 minutes. During the interval the room air sometimes underwent a slight change in its moisture content, but such change was invariably small when compared to the changes of vapour pressure and humidity experienced by the air current on passing through the soil in the tube C.

A decrease in the vapour pressure by 1 mm. of mercury corresponds to a loss of 10^{-6} grm. of water vapour per cubic centimetre or to one grm. of water vapour per cubic metre of air. If we plot the values of vapour pressure against time we shall be able to calculate from the area of the curve and the velocity of the air current in cubic centimetres per second, the total mass of water vapour extracted from the air current by the soil. For example, if A is the area of the curve when the vapour pressure is expressed in mm. of mercury and time in minutes and if V is the volume of air in c.c. entering the tube C per second, the mass of water vapour absorbed in grms. is given by :

$$A \times 60 \times V \times 10^{-6}.$$

TABLE XX

Name of soil	Wet bulb temperature in °C.			Humidity percentage			Vapour pressure (mm. of Hg.)			Time taken for attaining minimum value in minutes (t_1)	Time taken for completely exhausting the drying power of the soil in minutes (t_2)	Moisture gained by soil			Velocity of air current in cubic centimetres per second	Total volume in cubic metres of air passed over soil during the experiment ($V \times t_2$)	Mass of water lost by the air (actual dehydration) (grm.)	Amount of dehydration of air expressed in grammes per cubic metre	Mass of soil required to produce the observed dehydration in one cubic metre of air (grms.)
	Initial value	Minimum value	Maximum decrease caused by soil	Initial value	Minimum value	Maximum decrease caused by soil	Initial value	Minimum value	Maximum decrease caused by soil			Dry weight (M) of soil in grammes	Mass of water (m) absorbed in grammes	Expressed as percentage of dry weight of soil					
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)
Poona . . .	14·5	8·9	5·6	37	7	30	8·0	1·5	6·5	6·0	90	11·380	0·650	5·7	43·8	·236	0·730	3·09	48·12
Jalgaon . .	15·1	10·2	4·9	32	7	25	7·8	1·8	6·0	5·0	80	11·710	0·570	4·9	43·8	·210	0·620	2·95	55·71
Bangalore . .	14·1	11·4	2·7	28	15	13	6·6	3·5	3·1	2·0	10	11·370	0·030	0·3	43·8	·026	0·030	1·14	379·00
Lyallpur . .	14·0	11·7	2·3	26	14	12	6·3	3·5	2·8	1·5	8	11·400	0·027	0·2	43·8	·021	0·027	1·29	542·86
Sorbsil . . .	17·7	13·3	4·4	27	9	18	8·8	2·9	5·9	3·0	150	12·00	1·430	11·7	43·8	·394	1·740	4·41	30·44

This value can be compared with the actual increase in weight of the soil at the end of the experiment.

The vapour pressure-time curves are shown in Fig. 10. Sorbsil is an efficient absorber, and retains its power to absorb for the longest time. Next in order come the soils from Poona, Jalgaon, Bangalore and Lyallpur, respectively. The last two cause only a small depression in the wet-bulb temperature and the vapour pressure, and, even so, get exhausted within ten minutes. In all cases the dehydration of the air current is rapid and the vapour pressure attains its minimum value within a few minutes after the commencement of the experiment ; thereafter the vapour pressure slowly increases and becomes equal to that of the air outside.

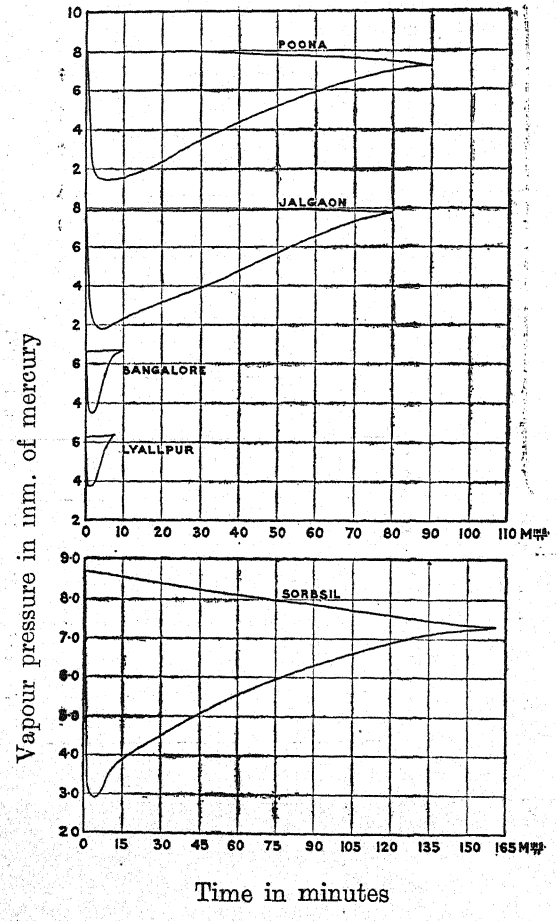


FIG. 10. Curves showing the variation of vapour pressure with time in the case of different soils and sorbsil; the straight lines at the top of each curve shows the vapour pressure in the room air outside the apparatus.

The results which may be deduced from Tables XV to XIX and from the curves in Fig. 10 are summarised in Table XX where, for facility of reference, the columns are numbered serially. Columns (2) to (10) give the initial and minimum values as well as the differences between these values in the case of the wet-bulb temperature, humidity percentage and the pressure of water vapour, in the experiments with the four soils and with sorbsil. Column (11) gives t_1 the time taken for attaining the minimum value and column (12) gives t_2 the total time taken for completely exhausting the absorbing power of each of the above substances. Column (13) gives the actual mass M of the substances used in the different experiments; this is usually between eleven and twelve grammes. Column (14) gives the mass of water vapour m absorbed in t_2 minutes. Column (15) gives the value of $\frac{m}{M} \times 100$. Column (16) gives the velocity V, of the air current which was adjusted to be 43·8 c.c. per second in all the experiments. Column (17) indicates the total volume of air which passed through the tube C in t_2 minutes. This volume is expressed in cubic metres. The next column (18) gives the mass of water lost by the air as calculated from the curves in Fig. 10 ($A \times 60 \times V \times 10^{-6}$). These values may be compared with the values of m in column (14). There is fairly good agreement in the values. The mass of water lost by the air can also be expressed in grms. per cubic metre (column 19). In the last column (20) is given the mass of soil required to desiccate one cubic metre of air to the extent indicated in column (19). From column (20) we note that with about fifty grms. of dry Poona soil we can remove about three grms. of water from one cubic metre of damp air. These figures do suggest that the black cotton soils may be used with profit for keeping the air in an enclosed space dry. The cost involved would be negligible when compared to that of common drying agents like sulphuric acid, calcium chloride, phosphorous pentoxide, etc. Further experiments to make practical use of the above results are in contemplation.

The investigations described in the preceding sections are intended to provide a general survey of this subject. The soils have been studied more or less as they exist in nature and there is ample scope for further investigations with the various constituents of each soil which may be separated by mechanical as well as chemical analysis. It would also be interesting to carry on the investigations on the moisture-variation index with each soil in its own natural environment, *e.g.*, Pusa soil at Pusa, Lyallpur soil at Lyallpur and so on.

In conclusion the authors desire to express their best thanks to the Director-General of Observatories for facilities given at the laboratories of the Meteorological Office at Poona. This investigation was made in the Agricultural Meteorology Section (India Meteorological Department) financed by the Imperial Council of Agricultural Research.

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STUDIES ON SOIL-MOISTURE

I. MOVEMENT OF SOIL-MOISTURE UNDER FIELD CONDITIONS

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The relation between water and a complex body like soil is one of the most complicated problems in soil physics. These complications multiply in the field where the conditions are daily changing and where the meteorological conditions, over which no control is possible, influence the movement of soil-moisture to a very great extent.

Apart from the external factors the inherent characteristics of the soil are so very widely varying from field to field and from year to year in the same field that any mathematical explanation will be far from satisfactory. Notwithstanding this, the problem is so very interesting and important both from the theoretical and practical point of view that much attention is being paid by soil scientists from all quarters.

Briggs [1907] considered soil as a bundle of capillary tubes and introduced the capillary hypothesis to explain the upward and horizontal flow of water inside the soil. King [1889], Hall [1904], Cameron [1911], McGee, Leake [1905] and others hold that sub-soil water rises up to supply the requirements of the crop, the range varying from 200 ft. to a minimum of 10 ft.

Keen [1918] has shown that in the case of an ideal soil, sub-soil water may rise up to 150 ft. but the process is extremely slow. But what is true of an ideal soil is never so for an ordinary soil specially under field conditions.

But none could assert with any degree of certainty the height to which water can rise by capillarity. On the other hand, more recent workers—Alway, Tulaikow, Burr 1914] are of opinion that sub-soil water is never available to the crop,

unless the roots themselves travel down to avail the water accumulated underneath.

The mode of capillary rise of sub-soil water in nature is a controversial point. Leather [1908] assumes that capillarity starts from the surface and gradually goes down as evaporation proceeds during the dry months and extends only to a limited depth. While Alway and Mcdole [1917] point out that sub-soil water rises up but the process is so very slow that it is doubtful whether this moisture will be able to reach the roots of annuals in the course of a growing season. Sen [1930] has shown that the upward movement of water starts from the surface and does not extend beyond 3 ft. The authors also hold the same opinion with Leather and Sen [*loc. cit.*] and show that the upward movement starts from the surface and seldom extends beyond 21 in. in the case of the Dacca Farm Soil.

Although Bengal gets sufficient rains during the monsoon, the highland tract dries up hopelessly during the dry season rendering winter cultivation quite disappointing. The investigation has been undertaken with the object of finding out ways and means of retaining optimum moisture specially for *rabi* cultivation.

Before making any attempt at retaining the soil-moisture it was worth-while studying the movement of soil-moisture in Bengal where the water-table rises up within 10 ft. from the surface and often much less than that during the monsoon and where the dry season does not extend over six months.

The first attempt at making a systematic study of the movement of soil water was to find out the moisture-content of the soil *in situ* at different depths during the dry season. Soil was bored down to a depth of 30 in. taking 3 in. at a time and moisture estimated for each depth. In order to facilitate the comparison of the moisture-content at various depths at different periods of the dry season, the data obtained in these experiments may be evaluated by graphical interpolation. The moisture distribution curves are all more or less continuous and show no abrupt changes of direction. The nature of the curves suggests an empirical exponential equation of the type

$$Y = Ao\ (1 - b\, e^{-a\, x})$$

where Ao is the maximum moisture-content, Y is the moisture-content at the depth x and b and a are constants. So that, when

$$x = 0\ ,$$

$$Y = Ao\,(1 - b)$$

and when $x = \infty$ (infinity) *i.e.* at great depth, $Y = Ao$.

The maximum value of the moisture has been obtained from the actual values and the curves have been fitted by method of least square (Figs. 4 & 5).

The agreement in the observed and the calculated values is fairly good. Thus beyond a certain depth, the moisture remains constant whatever the depth might be.

Water is held by the soil in the interstitial spaces due to surface tension. The essential condition for any mathematical interpretation of the mode of distribution of a given amount of moisture is that the curvature at all air-water interfaces is the same. The curvature increases or decreases according as the moisture-content of the soil becomes low or high. If we assume that the soil particles are uniform in diameter and if the packing is uniform throughout, the curvature shall be the same at all points, that is, the water film surrounding each soil particle will be of uniform thickness. At saturation, the air-water boundary is plain and the curvature increases as the moisture decreases and hence proportionately a greater energy is necessary to remove the water from the soil. Experimental results lend support to the assumption. It has been found in this experiment and by other workers particularly by Leather that the loss of moisture per unit of time depends on the quantity of moisture present in the soil. As the water escapes from the surface, the curvature of the air-water interfaces increases so that the surface is always in a state of tension hence it sucks water from beneath in order to reduce the curvature to a minimum and so does the next layer and so on. Thus the capillarity starts from the surface. The curvature of the air-water interfaces gradually decreases with the depth resulting in decrease of suction-pressure and ultimately it becomes so small that the water it pulls up from the next layer is negligibly small. In other words the capillarity does not extend beyond a certain depth for all practical purposes. Though the moisture-content remains constant beyond 21 in., it is unfair to assume that there is no movement of water at all there. Movement of water actually takes place but the region is in dynamic equilibrium. This region may be called the "Equilibrium moisture zone". It will be found from Table II that the evaporation coefficient assumes a negligible value at the equilibrium moisture zone. This is due to the fact that the soil there is saturated with water up to its field capacity so that the soil atmosphere above it contains more water-vapour than the upper layer. Hence the evaporation of water from the soil at that layer is negligibly small. Any loss, however small, from this layer is compensated by the capillary rise from the layer below it. Thus a dynamic equilibrium is established.

During the dry months, moisture does leave soil and the loss depends upon the meteorological conditions, *i.e.*, temperature, humidity, velocity of wind, etc., but as it has not been possible to have these data, the following deductions have been made as if the evaporation takes place under uniform temperature and humid conditions.

Thus if V be the water-content of the soil at any time t, then

$$-\frac{dv}{dt} = Ka \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (i)$$

where 'a' is the fraction of the surface covered with water-film and K is a constant which varies with the depth. Since the spheres are all uniform, they will have uniform moisture film surrounding them, that is, the thickness of the film will be

the same everywhere. Therefore 'a' is proportional to V. Substituting V for 'a' we have,

$$-\frac{dv}{dt} = K_1 V \quad \ldots \quad \text{(ii)}$$

i.e., the rate of loss of moisture $\frac{dv}{dt}$ is given as a product of V, the moisture-content and a constant K_1 which varies from layer to layer.

$$\frac{d \log V}{dt} = K_1 \quad \ldots \quad \text{(iii)}$$

Now V, the volume of the water actually found in the soil, is not all mobile, a part H (the residual moisture in the air-dry soil) cannot escape from the soil in the field under any circumstances hence while considering V, H must be deducted from it.

Thus substituting $(V—H)$ for V we have

$$\frac{d \log (V—H)}{dt} = K_1 \quad \ldots \quad \text{(iv)}$$

$$d \log (V—H) = K_1 \, dt$$

$$\log (V—H) = K_1 t + K_2 \quad \ldots \quad \text{(v)}$$

At a depth when K_1 assumes a negligible value,

$$\log (V—H) = K_2 = \text{constant.}$$

The above expression is a true measure of the moisture-content of the soil at any depth upto the limit of the experiment. The expression V involves two constants, K_1 and K_2 which require further discussion. The constant K_2 is evidently an arbitrary constant while K_1 is the coefficient of evaporation. It has already been stated that the moisture-content of the soil increases with the depth and assumes a constant value beyond 21 in. Apparently, the effect of evaporation beyond 21 in. is negligibly small. Hence, theoretically, K_1 should gradually decrease up to a certain depth, beyond which it becomes zero or less than any assignable quantity, while the arbitrary constant K_2 should gradually increase to a maximum constant value.

Experimental

Three sub-plots (18 ft. × 10 ft.) were selected from the experimental plot of the Dacca Farm high land area. The plots were ploughed, weeded off, laddered and kept undisturbed for the whole period of dry season. Before starting the experiment, eight points were marked off in each sub-plot.

Soil samples were collected from the plots up to a depth of 30 in. taking 3 in. of soil at a time. Each boring was thoroughly mixed up in a covered aluminium pot and about 30-40 grms. of soil were taken in a stoppered weighing bottle and weighed in the field. These samples were collected periodically at an interval of

three to four weeks, care being taken that the distance between the respective borings was never less than 3 ft. The samples were then taken to the Laboratory where it was dried at 105°C.—110°C., cooled in desiccator, weighed and moisture calculated on dry basis.

TABLE I

Total moisture in soil in situ *at varying depths—Dacca Farm*

Plot No. 12

Depth in inches	Moisture percentage 7th December 1932	Moisture percentage 28th December 1932	Moisture percentage 20th January 1933	Moisture percentage 17th February 1933	Moisture percentage 23rd March 1933	Moisture percentage 5th April 1933
0—3 . .	15·69	12·24	10·38	6·48	4·83	2·89
3—6 . .	17·25	17·84	13·55	11·90	10·56	7·63
6—9 . .	18·25	16·75	18·35	13·80	12·48	10·08
9—12 . .	20·31	18·88	20·21	17·00	16·28	12·42
12—15 . .	20·68	20.79	21·39	19·01	16·37	15·21
15—18 . .	22·38	22·08	22·26	20·00*	18·68	19·88
18—21 . .	22·90*	24·36	22·81	22·31	22·55	22·12
21—24 . .	23·42	24·94	24·50*	21·80	22·06	..
24—27 . .	23·69	24·04	25·30	22·22	22·24	..
27—30 . .	23·84	24·00	25·51	22·45	21·24	..

* The figures marked with asterisks have been obtained by interpolation.

Total moisture in soil in situ *at varying depths—Dacca Farm*

Plot No. 23

Depth in inches	Moisture percentage 28th December 1932	Moisture percentage 17th January 1933	Moisture percentage 17th February 1933	Moisture percentage 9th March 1933	Moisture percentage 5th April 1933
0—3 . .	11·31	8·82	6·80	5·93	4·44
3—6 . .	15·01	13·63	12·62	11·83	10·50
6—9 . .	18·32	15·64	15·20	15·73	14·66
9—12 . .	19·44	19·66	18·10	18·24	17·25
12—15 . .	22·88	22·18	21·23	19·87	19·38
15—18 . .	24·28	23·39	23·15	22·94	21·06
18—21 . .	24·23	24·36	23·33	23·10	22·00
21—24 . .	24·82	23·91	23·99	23·00*	23·24
24—27 . .	24·50	24·32	23·73	22·95	23·55
27—30 . .	23·48	23·41	23·17	23·25	22·35

* The figures marked with asterisks have been obtained by interpolation.

Total moisture in soil in situ *at varying depths—Dacca Farm*

Plot No. 30

Depth in inches	Moisture percentage 8th December 1932	Moisture percentage 7th January 1933	Moisture percentage 3rd February 1933	Moisture percentage 4th March 1933	Moisture percentage 23rd March 1933	Moisture percentage 8th April 1933
0—3 . .	15·60	9·21	8·48	5·81	4·06	3.38
3—6 . .	17·65	11·60	12·66	10·12	11·17	8·37
6—9 . .	18·51	14·28	12·30	12·85	13·08	12·59
9—12 . .	22·40	18·43	20·22	17·62	16·84	18·71
12—15 . .	24·67	19·52	21·09	21·40	20·56	21·21
15—18 . .	25·09	19·28	21·93	22·09	21·22	22·80
18—21 . .	25·69	20·72	22·66	20·17	22·46	22·57
21—24 . .	25·69	22·42	23·00	23·51	21·74	22·30
24—27 . .	24·44	21·68	22·52	23·61	21·47	22·54
27—30 . .	24·54	22·14	23·12	23·61	22·22	22·78

The surface layer being directly influenced by the sun, the wind and other meteorological changes dries up very quickly and as such suffers maximum loss. This loss of moisture decreases with the depth and then becomes constant (Figs. 1, 2 and 3). These curves are similar to those of Greene [1928]. The graphs bring out some interesting features. In the first place the graphs are all smooth and tend to converge to a depth of about 21 in. then diverge almost parallel to the axis of depth showing that the soil profile is uniform up to the depth considered and beyond that depth the moisture-content remains constant. The steepest curve is obtained at the end of the dry season while the flattest at the beginning, thus indicating that the effect of evaporation is perceptible only up to a depth of a few inches.

Plot No. 12.

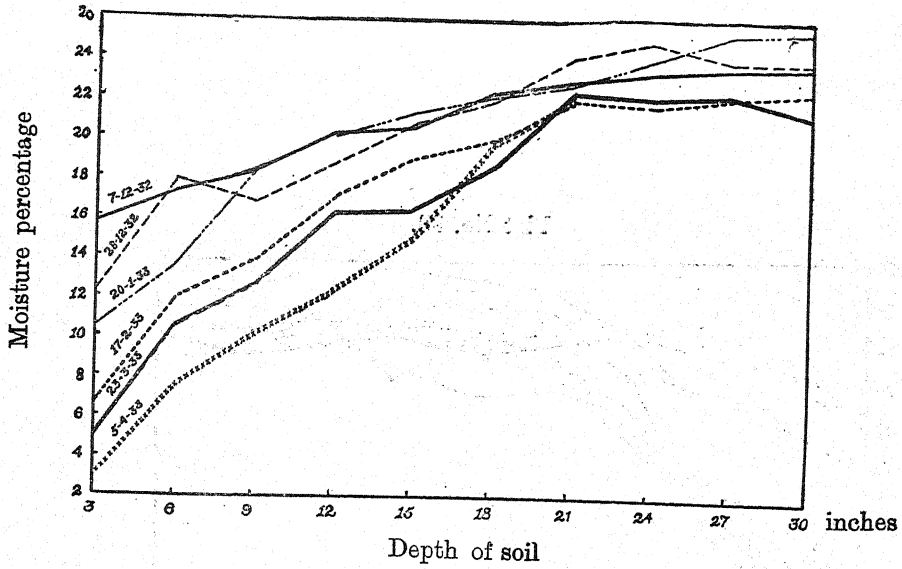

Fig. 1.

Plot No. 23.

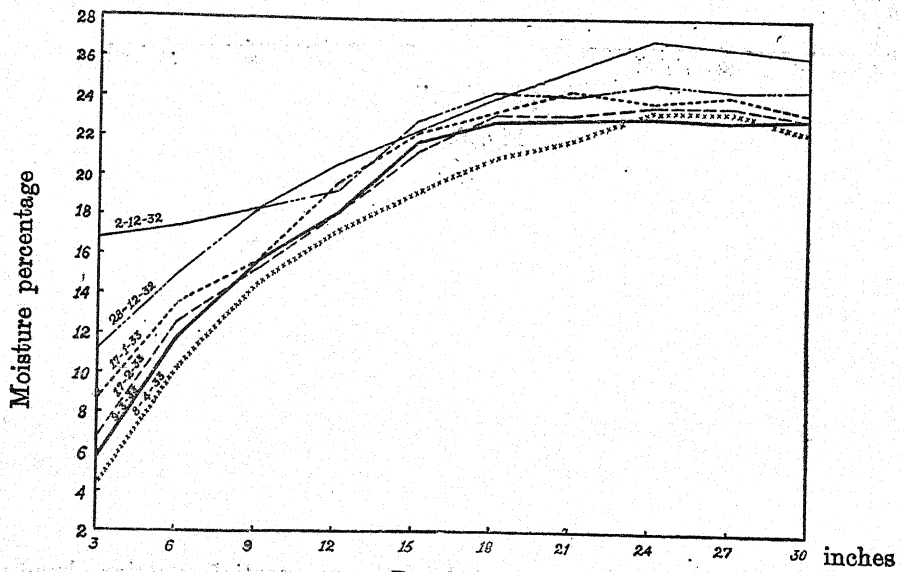

Fig. 2.

Plot No. 30.

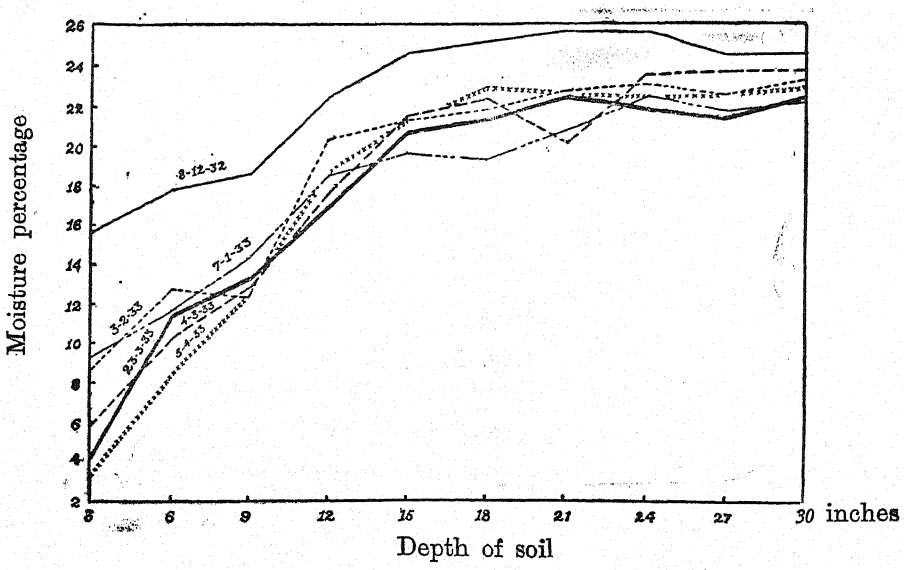

Fig. 3.

The above curves may be represented by the exponential equation already discussed—two curves are shown here (Figs. 4 and 5).

Curve No. 1.

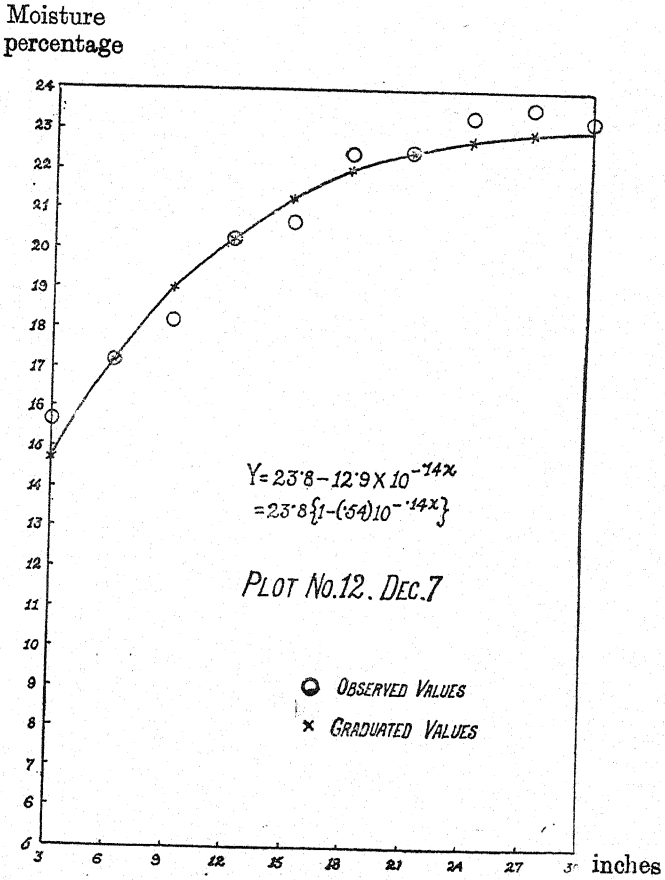

Fig. 4.

Curve No. 2.

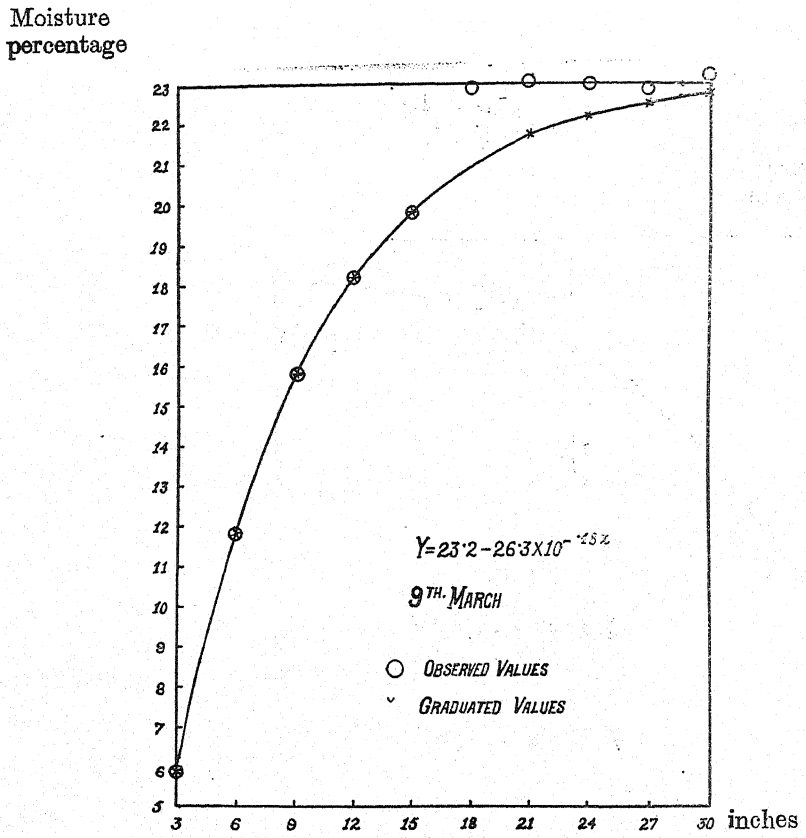

Fig. 5.

The above equation is purely an empirical one and has got no theoretical bearing. Since the soil profiles are scarcely uniform the moisture content will depend upon the physical nature of the soil.

An inspection of the figures in Table I, shows that evaporation proceeds in every layer of the soil *in situ* during the dry months resulting in the decrease of the moisture per cent in all the layers up to the "Equilibrium-moisture-zone." The data can be correctly fitted up into the evaporation equation (v). On plotting log $(V-H)$ against t (t being recorded in days), it is found that all the points lie about a straight line (Figs. 6, 7 and 8).

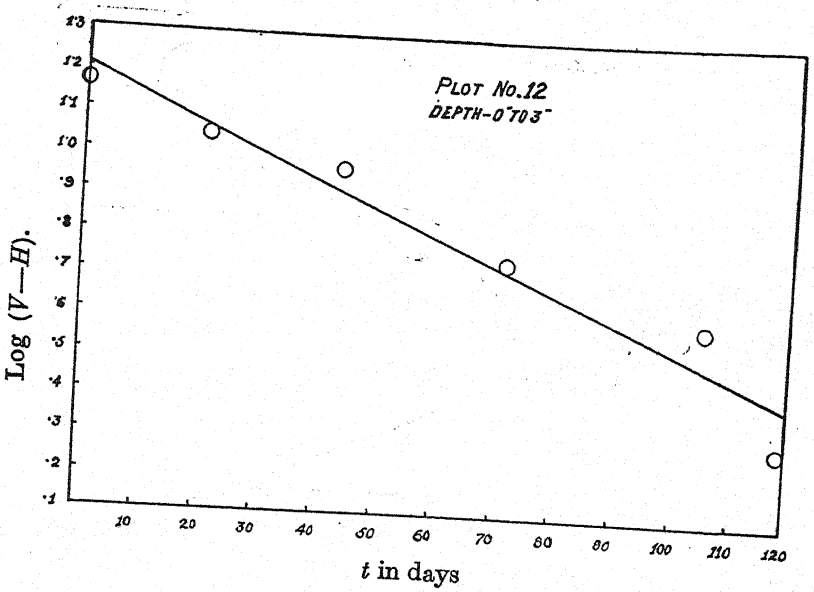
PLOT No.12
DEPTH-0"TO3"
Log (V—H).
t in days

Fig. 6.

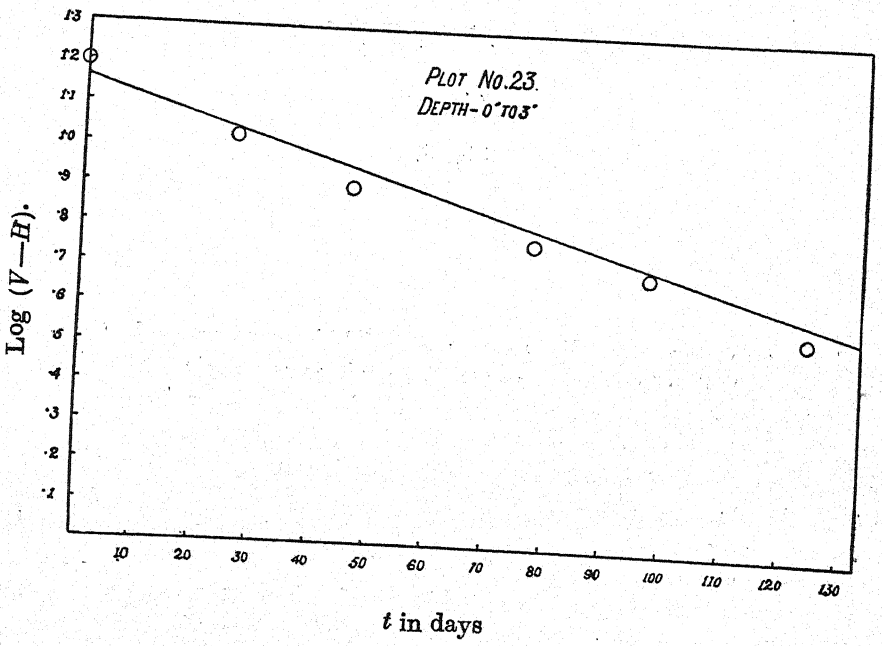
PLOT No.23.
DEPTH-0"TO3"
Log (V—H).
t in days

Fig. 7.

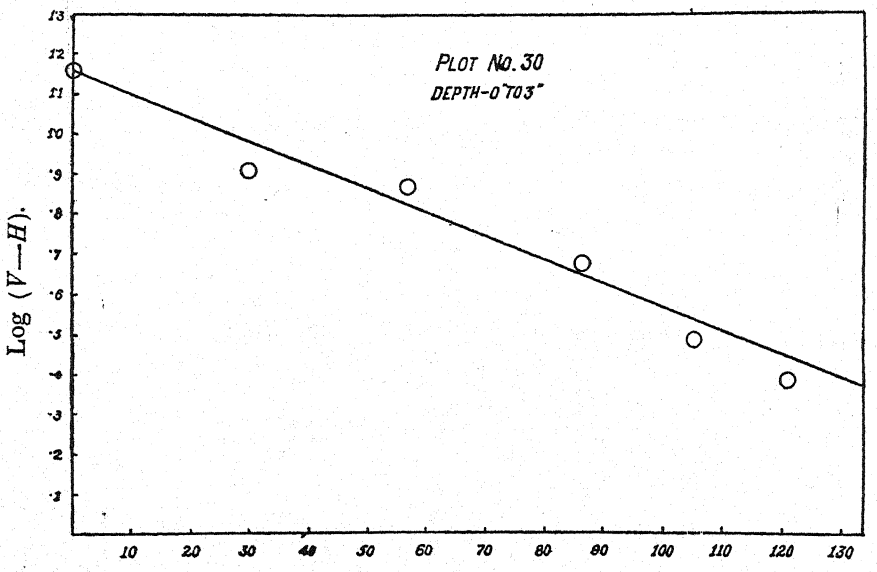

Fig. 8.

These graphs are all of different slopes which decrease as the depth increases until at the " Equilibrium-moisture-zone ", they become practically parallel to the axis of time which lends further support to the fact that the moisture-content remains practically constant beyond a certain depth, *i.e.* beyond the " Equilibrium-moisture-zone " and further that the capillarity does not extend beyond a certain depth. The linear equation (v) being the true measure of the moisture-content of the soil, the value of the constants may be of some interest. They have been worked out from the experimental data. A glance at Table II will show that the evaporation constant K_1 decreases with the depth and reaches zero at infinite depth but its value beyond 21 in. is negligibly small and may be taken to be zero for all practical purposes and log K_2 approaches its maximum constant value.

TABLE II

Table for the constants

	$-K_1$	K_2
Plot No. 12.		
0″—3″	0·006743	1·2110
3″—6″	0·003091	1·2453
6″—9″	0·001511	1·2141
9″—12″	0·001746	1·2983
12″—15″	0·001421	1·3116
15″—18″	0·0007803	1·3281
18″—21″	0·001268	1·3299
21″—24″	0·001044	1·3794
24″—27″	0·0005704	1·35702
27″—30″	0·0006016	1·3583
Plot No. 23		
0″—3″	0·005059	1·1670
3″—6″	0·001625	1·1469
6″—9″	0·0008029	1·2071
9″—12″	0·0006208	1·2564
12″—15″	0·0008666	1·3236
15″—18″	0·0006420	1·3539
18″—21″	0·0004882	1·3501
21″—24″	0·0003175	1·3519
24″—27″	0·000255	1·3481
27″—30″	0·000135	1·3270
Plot No. 30		
0″—3″	0·006179	1·1656
3″—6″	0·002264	1·1716
6″—9″	0·000985	1·1664
9″—12″	0·00077	1·2889
12″—15″	0·0003574	1·3123
15″—18″	0·0001398	1·3164
18″—21″	0·000351	1·3275
21″—24″	0·0004539	1·3558
24″—27″	0·0002208	1·3277
27″—30″	0·0002037	1·3302

It will be observed from the above that certain discretion had to be applied in representing the moisture-content by the regression equation (v), hence it is worthwhile to submit the values of the constants to a more rigorous test. The values of K_1 and log K_2 in the table have been calculated from equation (v). The high correlation coefficient ($r = 0·97$) between the observed and the calculated

values leaves no doubt as to the validity of the deductions applied under field conditions. The slight discrepancy in the result is not surprising in view of the various sources of error involved in the experiment and numerous assumptions made in the deduction.

The following table shows the observed and the calculated values of log (V—H) of the 1st layer (0″-3″).

TABLE III

Plot No. 12	
log (V—H) observed	Log (V—H) calculated
1·1690	1·2110
1·0554	1·0694
0·9754	0·9144
0·7442	0·7256
0·5910	0·4963
0·2920	0·4086
Plot No. 30	
log (V—H) observed	Log (V—H) calculated
1·1664	1·1656
0·9190	0·9802
0·8779	0·8135
0·6842	0·6342
0·4913	0·5166
0·3890	0·4179
Plot No. 23	
log (V—H) observed	Log (V—H) calculated
1·2041	1·2110
1·0162	1·0355
0·8940	0·9343
0·7686	0·7775
0·6989	0·6763
0·5453	0·5397

Similar agreements were obtained for other depths also.

EFFECT OF RAINFALL

If there be any rainfall during the tenure of the experiment, the entire course of moisture movement will be disturbed and as such any deduction be erroneous. If the rainfall be sufficiently heavy as to accumulate a good deal of water on the surface then the depth to which this water will penetrate by percolation will depend upon the equilibrium-moisture holding capacity of the soil which in the case of the Dacca Farm soil is about twenty-three per cent, so the amount of water that will be absorbed by the soil will be the difference between the equilibrium-moisture holding capacity and the actual moisture-content of the soil.

But if the rainfall be not sufficient to saturate all the soil above the Equilibrium-moisture-zone so as to raise it to the equilibrium-moisture-content then the

water will travel only to a limited depth thus increasing the moisture content of the upper layers. The surface being more susceptible to evaporation, dries up quickly, scarcely leaving any opportunity to detect the increase of moistuie, unless the observation is made shortly after, while the intermediate layers show a definite increase in the moisture per cent.

Though the rainfall was very insignificant during the 1st stage of experiment, the difference between the successive periods indicates clearly the departure from the normal process of drying.

TABLE IV

Moisture after rainfall

Depth	Plot No. 23 Moisture percentage 25th May	Plot No. 12 Moisture percentage 15th May	Plot No. 30 Moisture percentage 26th May
0″—3″	18·35	27·07	15·45
3″—6″	18·35	23·25	18·95
6″—9″	14·87	22·20	19·05
9″—12″	16·05	21·80	21·55
12″—15″	20·15	21·88	22·42
15″—18″	20·65	24·51	21·42
18″—21″	22·58	18·96	24·50
21″—24″	26·15	21·81	27·93
24″—27″	26·45	22·28	23·40
27″—30″	24·80	22·30	22·65

Rainfall	In.
November 1932	0
December 1932	0
January 1933—	
22nd	0·05
25th	0·03
February 1933—	
13th	0·8
19th	0·11
March 1933	0
April 1933—	
14th	2·70
15th	0·45
16th	1·32
17th	1·28
18th	..
19th	0·6
20th	0·36
23rd	2·65
Total	9·36

Rainfall	In.
May 1933—	
1st	0·44
5th	0·05
6th	1·06
9th	1·22
10th	0·02
11th	0·04
12th	0·40
18th	1·28
22nd	0·35
23rd	0·57
25th	0·47
30th	1·80
Total	7·70

It will be seen from the above tables that there had been abundant rainfall during the months of April and May to accumulate a good deal of water so as to allow percolation down to the lower layers or even to the water-table below. Table IV shows that though the rainfall was heavy, the moisture-content of the soil in any layer never exceeded twenty-three per cent which is the Equilibrium-moisture holding capacity of the soil under investigation. Hence it follows that a portion of the rain water was used up in raising the soil of the upper layers to its equilibrium-moisture-content and the excess water percolated down to the lower layers.

But as a matter of fact winter rainfall in Bengal is scarcely sufficient to moisten the soil to any depth hence the considerations can seldom be useful. This is, however, of importance where irrigation water is concerned. The supply of too much water for irrigation purposes is simply to run it to waste.

In conclusion, the authors take the opportunity of expressing their indebtedness to the Imperial Council of Agricultural Research for the financial grant towards the cost of the investigation.

Our acknowledgments are due to Messrs. K. McLean, B.Sc., Director of Agriculture, Bengal and to Gostabehari Pal, M.Sc., Agricultural Chemist, Bengal, for the kind interest they took in the investigation.

Summary

1. The condition of soil-moisture *in situ* at varying depths have been studied in the Dacca Farm (highland) during the dry season. The loss of soil-moisture is greatest and very rapid in the 1st three inches then gradually decreases up to a depth of 18 in. Beyond 18 in. there is an "Equilibrium-moisture-zone" which remains at the same moisture-content up to the end of the dry season. The moisture-content at this zone is about twenty-three per cent.

2. Beyond this depth of 21 in. up to 30 in. the soil-moisture remains constant. This may be a possible explanation as to why the deep-rooted crops can resist drought.

3. The phenomena of capillary rise of water in soil has been discussed and it has been shown that the upward movement of water in soil starts from the surface and gradually goes down.

4. The evaporation of soil-moisture under field condition can be satisfactorily expressed by the formula

$$\log (V-H) = K_1 t + \log K_2.$$

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MINERAL MATTER IN THE JUICE OF SUGARCANE AND ITS EFFECT ON THE RECOVERY OF WHITE SUGAR, I

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The sugar industry in India received considerable impetus after the imposition in 1932 of custom duty on white sugar imported into India, which led both in the Punjab and other provinces to the establishment of a considerable number of sugar factories. Prior to this date only a few refineries were working in the Punjab and manufactured white sugar from *gur* or jaggery imported from the United Provinces. These imported *gurs* were generally impure and soft in consistency, sometimes resembling dark-coloured, semi-viscous fluid but were invariably preferred to the *gurs* produced in the Punjab which were apparently cleaner and more attractive in appearance. The reason assigned for this partiality lay mainly in the alleged greater recovery of white sugar from the United Provinces *gurs*, and this was assisted by their lower price even after allowing for freight and other charges. The analyses for sugar content of a large number of Punjab *gurs* do not reveal any striking differences from the *gurs* produced in the United Provinces, although it has been suggested that the method of making *gur* as practised in the Punjab differs from the method in vogue in the United Provinces, in so far that in the former the juice is kept constantly stirred with a *mashad* or ladle not only during concentration but even after the thickened syrup has been transferred to the *gand* for solidification. It was suggested that this continual stirring tends greatly to reduce the size of the crystals in the *gur*, which would otherwise remain fairly large, and that this is responsible for the lower recovery of white sugar from Punjab *gurs*.

In order to ascertain the correctness of this view, it was decided to prepare *gurs* from the same variety of cane by both methods for analysis. That prepared by the United Provinces method took longer to solidify in the pan, was darker in colour and would not easily crumble and was so sticky that it could be drawn out into threads. In spite of these different physical features, the analytical data given in Table I did not show any difference which would account for these facts.

TABLE I

Analysis of gurs *prepared by the United Provinces and the Punjab methods*

Variety	Sugar in juice		Sugar in *gurs* prepared according to					
			U. P. method			Punjab method		
	Sucrose Per cent	Glucose Per cent	Sucrose Per cent	Glucose Per cent	Total sugar	Sucrose Per cent	Glucose Per cent	Total sugar
Co. 205 .	12·57	1·56	66·17	8·77	74·94	65·92	9·31	75·23
Co. 213 .	16·53	0·67	75·74	6·12	81·86	75·24	6·12	81·36
Co. 223 .	16·45	0·72	77·26	6·53	83·79	75·24	6·12	81·36

Some further analyses were undertaken in order to ascertain if differences in the climate and soil conditions in the United Provinces and in the Punjab respectively might account for any differences found in the sugars of the same cane varieties which might ultimately be reflected in the quality of the *gurs* produced from the two provinces. The data obtained are shown in Table II and, with the exception of Co. 290, did not show any pronounced difference in the sucrose and glucose content of some of the main varieties of sugarcane common to both provinces. It seems reasonable then to look in other directions for the alleged inferiority of the Punjab *gurs*.

TABLE II

Showing comparative analytical data for the same varieties of cane grown in the United Provinces (Shahjahanpur) and the Punjab (Jullundur)

Variety	Province	Juice	Sucrose per cent on cane	Glucose
Co. 213 . . .	U. P. . .	63·0	10·1	0·66
,, . . .	Punjab .	64·8	10·0	0·33
Co. 285 . . .	U. P. . .	66·0	9·1	..
,, . . .	Punjab .	62·5	9·5	0·18
Co. 293 . . .	U. P. . .	66·3	12·4	0·53
,, . . .	Punjab .	67·9	9·6	0·39
Co. 300 . . .	U. P. . .	65·3	12·4	0·36
,, . . .	Punjab .	67·2	11·3	0·26
Co. 312 . . .	U. P. . .	63·7	10·9	0·48
,, . . .	Punjab .	68·4	10·8	0·28
Co. 313 . . .	U. P. .	63·0	11·6	0·44
. . .	Punjab .	67·1	11·7	0·25

In sugar refineries the 'nett rendiment' values are used for determining the quality of raw sugars and are also employed as a check on the amount of white sugar actually obtained against the amount expected. This value takes into consideration the amount of sucrose, glucose and the ash or mineral matter present in the raw sugar and is calculated from the following formula :—

$$R = \text{Polarisation} - (I \times \text{glucose per cent}) - (X \times \text{ash per cent})$$

The value of X in the above equation varies between 3·5 and 5 for different kinds and quantities of raw sugar produced in different localities and countries.

It is clear from the above that all the sucrose actually present in the raw sugar cannot be obtained as white sugar and that the amount left uncrystallised in the molasses is equal to the amount of reducing sugars + X times the amount of ash. The mineral matter, therefore, is of far greater account than the glucose content. The figures of analyses for *gurs* purchased from the markets in some of the important sugar producing centres are given in Table III.

TABLE III

Analytical data for gurs *from the Punjab and the United Provinces*

Description of sample	Polarisation or sucrose	Glucose	Ash	Nett rendiment
		Punjab		
Lyallpur . .	73·70	7·20	4·47	50·9
Jullundur . .	69·54	8·18	5·43	42·4
Amritsar . .	72·80	10·50	4·34	47·1
Sonepat . . .	66·43	11·32	5·49	36·0
Gojra (Lyallpur) .	71·40	7·90	6·00	42·5
AVERAGE . .	70·77	9·02	5·15	43·8
		United Provinces		
Gorakhpur . .	70·32	13·53	2·86	46·78
Do. . .	77·09	12·00	3·27	53·64
Do. . .	68·80	18·00	2·53	41·94
Muzaffarnagar .	74·00	11·70	2·53	53·40
Do. . .	70·00	13·20	2·38	48·50
AVERAGE .	72·04	13·69	2·71	48·85

It will be seen that the amount of sucrose in the Punjab *gurs* is practically the same as in *gurs* from the United Provinces, while the figures for glucose are decidedly lower. This means that the method of *gur*-making as practised in the Punjab is not inferior to the U. P. method, since any defect in the Punjab method would have resulted in inverting the sucrose and thus raising the glucose content. The main reason for the inferiority of the Punjab *gurs* is apparently their high mineral content, which is almost double that of the U. P. *gurs*.

This fact led to a systematic enquiry being undertaken into the causes responsible for the high ash content of the *gurs* manufactured in this province. The investigation has been carried on for the last three years and the results obtained so far are presented in this paper.

Apart from any adhering dust or soil particles which may gain access to the juice during the crushing of sugarcane, all mineral matter found in the *gur* can be traced to the soluble salts present in the juice from which the *gur* was prepared. This investigation, therefore, resolved itself into a study of the ash content of the juice from different varieties of cane grown in different localities.

During the year 1932-33, the Punjab possessed the second largest area under sugarcane—namely, 558,152 acres—of any province in India, the United Provinces standing first with 1,773,211 acres in the same year. This area must necessarily increase if the extra demand of new sugar factories, which have recently come into being, is to be met. The districts of Jullundur and Hoshiarpur comprise a tract which has for long been regarded as one of the most suitable for the production of sugar, although, from the climatic point of view, the south-eastern districts of Karnal and Rohtak appear to be better.

With the development of scientific methods in agriculture and the introduction of new varieties of sugarcane, other tracts such as the new colony areas, have also taken up the cultivation of this crop. It seems, therefore, that in spite of the unfavourable climatic conditions for the growth of sugarcane and competition with more paying crops such as cotton, the Punjab is likely to remain an important sugar-producing province. Punjab *gurs* give about five per cent less white sugar than the U. P. *gurs*, which means a great loss to the province and it is, therefore of importance to ascertain the causes responsible for the low quality of the canes alleged to yield inferior *gurs*.

The lines on which the investigation has been carried out are :—

1. Classifying sugarcane varieties according to the ash content of their juice.
2. Classifying different tracts in the province with reference to the ash content of the juice of sugarcane grown therein.
3. Studying the relationship that may exist between the ash content of sugarcane juice and the composition of the soil.
4. Investigating the possibilities of controlling the factors responsible for the high ash content of sugarcane juice in the Punjab.

1. Mineral content of different varieties of sugarcane grown in the Punjab

Table IV gives the results of analyses of different varieties of sugarcane from localities in different parts of the province. The figures for any one variety are generally the mean of several figures relating to that variety and were obtained after the cane had reached maturity. It is possible that the canes obtained from different localities might have been influenced by differences in climatic conditions

or in manurial treatments, but it will be seen that the mineral matter in cane varies from 0·24 per cent to 0·70 per cent only. The value of 0·24 for Co. 252 and Co. 300 refers to samples of these varieties obtained from Gurdaspur only, but as will be seen later, all varieties grown in this district show a very low ash content. Most of the varieties examined (12 out of 18) had an ash content in the neighbourhood of 0·50 per cent and only four contain more than 0·60 per cent. This difference of 0·1 per cent will affect the recovery of sucrose by only 0·4 per cent at the maximum, if these were the only varieties available to any particular factory, which is a very remote contingency.

TABLE IV

Analytical data for some of the important varieties of sugarcane grown in the Punjab

(Average figures)

Variety	Juice	Sucrose	Glucose	Total sugar	Total solids	Ash	Glucose ratio	Purity coefficient	Nett rendiment
Co.205	58·9	7·90	0·61	8·51	10·77	0·53	7·7	74·5	5·5
Co.213	67·4	9·45	0·41	9·86	11·71	0·55	4·4	80·7	7·1
Co.223	64·4	8·70	0·57	9·27	11·21	0·43	6·8	76·1	6·8
Co.244	66·1	9·55	0·41	9·96	11·93	0·67	5·4	78·6	6·8
Co.252	66·4	6·70	1·22	7·92	9·20	0·24	18·2	72·2	4·6
Co.270	64·9	9·60	0·39	9·99	12·15	0·46	4·4	80·0	7·6
Co.272	66·5	7·50	1·29	8·79	10·00	0·43	20·6	74·4	4·7
Co.281	68·5	10·92	0·21	11·13	13·28	0·53	2·4	82·4	8·8
Co.285	62·7	9·99	0·31	9·30	11·49	0·60	3·8	78·2	6·6
Co.287	64·2	8·50	0·80	9·30	11·01	0·46	9·4	77·6	6·0
Co.290	69·5	10·01	0·26	10·27	12·17	0·61	3·1	81·7	7·6
Co.300	69·0	9·20	1·00	10·20	11·00	0·24	10·9	83·6	7·4
Co.312	69·9	11·20	0·20	11·40	13·15	0·69	1·8	85·0	8·6
Co.313	68·1	11·10	0·27	11·35	13·00	0·55	1·4	85·6	8·9
Co.356	62·9	9·20	0·36	9·60	11·20	0·70	3·9	82·0	6·4
Katha	59·3	8·40	0·43	8·83	10·91	0·58	5·9	77·0	4·9
Suretha	60·0	9·08	0·19	9·27	11·30	0·52	7·1	80·1	2·1
Kansar	59·4	7·37	0·93	8·30	11·23	0·78	3·7	65·5	12·7

As pointed out by Thieme in 1930, it has been noticed in the Java factories that since 1915 the ash content of their cane juices has been increasing, particularly during the years 1926-29—a period which coincided with the production of P. O. J. 2878 cane. It appears that a high ratio of ash to non-sugars is a characteristic of modern canes, resulting in lower yields in the factory. In the case of the Punjab, however, taking the province as a whole, no striking differences in the mineral content of juice from different varieties at present available are noticeable.

It must be remembered, however, that where a number of varieties is available which shows highly favourable characters, such as high yields, high purity figures and low glucose ratios, those which have a low ash content must invariably be preferred and in propagating canes suitable for cultivation in the Punjab, this

question of ash content must receive full consideration especially in view of the fact that cane in the Punjab is liable to experience unfavourable climatic conditions.

2. Effect of soil and climatic conditions in different localities on the mineral content of the cane juice

Table IV gives the results obtained during the first year of the enquiry; eighteen varieties were examined from eight different districts, *viz.*, Hansi, Rohtak, Jullundur, Gujranwala, Lyallpur, Sargodha, Montgomery and Multan. In addition to the ordinary analyses of the juice, the amount of mineral matter was also determined in sixty-six samples. It is not proposed to give the analysis of each one of these separately as in the first year of the enquiry there was some difficulty in obtaining samples of certain varieties from all the localities at the same comparable period of growth. It was, therefore, decided to continue the investigation in the following year, under more comparable and better controlled conditions. The preliminary results obtained during the first year indicate that the province can be divided into three regions, *viz.*, sub-montane, central and western districts in relation to the amount of mineral matter present in the juice of sugarcane grown in different tracts.

In order to ascertain if any relationship exists between the amount of mineral matter in the cane juice and the amount of water-soluble salts present in the soil on which the cane grew, thirty-two soil samples were examined for total solids, carbonates, bicarbonates, chlorides and sulphates in a ten per cent water-extract of soil. It was anticipated that the amount of mineral matter present in the juice would be related to the amount of water-soluble matter in the soil, but contrary to expectations, the analytical results lent no support to this hypothesis.

The work carried out during the second year confirms all the conclusions arrived at the previous year. Before describing the results obtained, it may be as well to summarise the conditions under which the enquiry was conducted :—

(*i*) Since it had been found in the first year that, with one or two exceptions, the differences in the mineral content of the different varieties were not so great as the differences due to geographical situation, only four varieties which were available at all the localities selected were reserved for examination, *viz.*, Co. 213, Co. 285, Co. 290 and Co. 300.

(*ii*) In order to eliminate possible differences in mineral content due to difference in age, the canes were analysed at approximately the same period of growth. Thus, with the exception of Co. 213, which is a late ripener and was analysed once only in the third week of February 1933, the other three varieties were analysed twice in the season, *i.e.*, once in the middle of December 1932, before the onset of frost and again during the 3rd week of February 1933, when the canes were expected to have recovered completely from the effect of frost.

(*iii*) The crops selected had received no special manurial or other treatment likely to produce artificial variations in the chemical composition of the soil.

(*iv*) The localities selected were typical of the different soil and climatic conditions of the province.

(*v*) The mineral matter was determined in the unclarified juice, so that nothing was added to or removed from it.

As part of the enquiry, Co. 285 and Co. 290 were kept under observation with the object of studying variations in the composition of the juice at different periods of growth. The analyses were carried out at monthly intervals beginning in November 1932 and extending to the end of February 1933 and the results obtained are given in Table V. It will be seen that after the cane had reached maturity, the juice or soluble mineral matter varied but little. Thus a difference of some days or even a few weeks in the analysis will not effect to any great extent the relative amount of mineral matter present in the juice of different varieties or in the same variety from different localities. This explains why the general conclusions drawn from the first year's work (when in the case of some of the varieties the analyses were carried out at an interval of 2 to 3 weeks) are in no way modified by the more systematic and better controlled work in the following year.

TABLE V

Variations in the composition of the juice of sugarcane at different stages of its growth

Date of analyses	Percentage on cane							
	Juice	Sucrose	Glucose	Total sugar	Total solids	Ash	Nett rendiment	Purity
				Co. 285				
1st Nov. 1933.	63·5	7·38	0·38	7·76	9·84	0·66	4·68	75·0
1st Dec. 1933.	62·6	8·34	0·26	8·60	10·74	0·68	5·71	78·0
1st Jan. 1934.	61·9	8·38	0·11	8·94	10·55	0·62	6·56	83·7
31st Jan. 1934	62·8	8·72	0·20	8·92	10·85	0·63	6·30	80·4
27th Feb. 1934	60·0	8·92	0·06	8·98	11·58	0·72	6·34	80·5
				Co. 290				
1st Nov. 1933.	65·7	5·67	0·96	6·63	8·42	0·61	2·59	67·0
1st Dec. 1933.	70·0	9·91	0·26	10·17	11·84	0·68	7·28	84·0
1st Jan. 1934.	68·4	9·28	0·56	9·84	11·20	0·71	6·22	83·0
31st Jan. 1934.	67·7	7·15	1·56	8·71	11·39	0·77	2·89	62·8
27th Feb. 1934.	64·4	8·89	0·60	9·49	11·41	0·62	6·12	77·9

The results of the analyses of similar canes from different localities are given in Tables VI to IX. It will be observed that the greatest amount of mineral matter in the juice is to be found in canes from Multan, and the least in those

from Gurdaspur and Rawalpindi. The 'nett rendiment' values for Rawalpindi canes, however, are very much lower than for those from Gurdaspur. The reason apparently is that cold weather, particularly when the temperature at night falls, for any prolonged period, below the freezing point, brings about inversion of the sucrose already formed, resulting in an increase in the amount of glucose or invert sugar which prevents a corresponding amount of sucrose from crystallizing during the preparation of white sugar. On the other hand, a comparison of the figures for mineral matter obtained in December and February clearly indicates that frost has not affected them.

TABLE VI

Analytical data for Co. 213 from different localities in the Punjab

Locality	Date of analysis	Juice per cent	Sucrose per cent	Glucose per cent	Total sugar per cent	Total solid per cent	Ash per cent	Nett rendiment	Glucose ratio	Purity
Lyallpur	21st Feb. 1934	67·0	9·21	0·30	9·51	11·84	0·60	6·81	3·2	78
Sargodha	19th Feb. 1934	68·5	9·39	0·39	9·78	11·88	0·50	7·25	4·2	79
Montgomery	Not available									
Multan	17th Feb. 1934	68·2	7·8	0·18	7·98	9·7	0·72	5·10	2·3	80
Sirsa	23rd Feb. 1934	63·8	9·6	0·17	9·77	11·7	0·50	7·68	1·8	82
Jullundur	19th Feb. 1934	67·0	9·9	0·31	10·21	12·1	0·51	7·80	3·2	82
Gurdaspur	25th Feb. 1934	65·4	11·09	0·13	11·22	12·75	0·45	9·38	1·2	87
Rawalpindi	22nd Feb. 1934	68·8	9·00	0·72	9·72	11·44	0·31	7·19	8·0	79

TABLE VII

Analytical data for Co. 285 from different localities in the Punjab

Locality	Date of analysis	Juice per cent	Sucrose per cent	Glucose per cent	Total sugar per cent	Total solid per cent	Ash per cent	Nett rendiment	Glucose ratio	Purity coefficient
Lyallpur	15th Dec. 1933	62·8	8·73	0·16	8·89	10·86	0·65	6·30	1·8	80
	21st Feb. 1934	63·4	9·56	0·07	9·63	12·03	0·69	7·07	0·7	80
Sargodha	16th Dec. 1933	62·8	8·89	0·35	9·24	10·9	0·64	6·30	4·0	82
	19th Feb. 1934	62·5	9·45	0·17	9·62	11·81	0·56	7·28	1·8	80
Montgomery	14th Dec. 1933	61·4	9·00	0·17	9·20	10·6	0·69	6·43	2·0	85
	18th Feb. 1934	64·4	9·70	0·17	9·87	11·9	0·74	7·44	1·8	82
Multan	16th Dec. 1933	62·3	8·80	0·10	8·90	10·7	0·85	5·71	1·1	82
	17th Feb. 1934	61·2	7·70	0·17	7·87	10·3	0·76	4·87	2·5	75
Hansi	15th Dec. 1933	57·4	7·90	0·36	8·30	9·8	0·53	5·78	4·6	81
	23rd Feb. 1934	60·0	9·20	0·20	9·40	11·5	0·51	7·21	2·8	80
Jullundur	13th Dec. 1933	62·0	8·10	0·63	8·70	10·8	0·37	6·17	7·7	75
	19th Feb. 1934	62·8	10·30	0·22	10·52	12·3	0·45	8·50	2·5	84
Gurdaspur	13th Dec. 1933	61·6	8·39	0·38	8·77	10·46	0·31	6·92	4·5	80
	25th Feb. 1934	61·9	10·20	0·22	10·42	11·90	0·41	8·54	2·2	86
Rawalpindi	15th Dec. 1933	63·3	6·41	1·07	7·48	9·31	0·39	3·98	16·6	68
	22nd Feb. 1934	62·7	7·40	0·96	8·36	10·52	0·43	5·89	13·0	70

TABLE VIII

Analytical data for Co. 290 from different localities in the Punjab

Locality	Date of analysis	Juice per cent	Sucrose per cent	Glucose per cent	Total sugar per cent	Total solid per cent	Ash per cent	Nett rendiment	Glucose ratio	Purity coefficient
Lyallpur	15th Dec. 1933	70·5	9·02	0·32	9·34	11·44	0·72	6·17	3·6	79
	21st Feb. 1934	67·6	8·47	0·84	9·31	12·40	0·65	5·35	9·9	68
Sargodha	16th Dec. 1933	68·3	10·3	0·29	10·60	11·60	0·57	8·01	2·8	89
	19th Feb. 1934	69·2	9·97	0·60	10·57	12·77	0·51	7·58	6·0	78
Montgomery	17th Dec. 1933	64·9	9·1	0·40	9·5	10·90	0·53	6·84	4·4	84
	18th Feb. 1934	62·1	9·1	0·40	9·50	11·21	0·55	6·77	4·4	81
Multan	Not available									
Hansi	15th Dec. 1933	63·7	9·5	0·22	9·70	11·02	0·60	7·20	2·3	86
	23rd Feb. 1934	70·0	9·3	0·23	9·53	13·00	0·70	6·62	2·8	72
Jullundur	19th Dec. 1933	71·0	11·5	0·65	12·20	13·03	0·26	9·94	5·8	88
	19th Feb. 1934	68·7	11·6	0·51	12·11	13·30	0·25	10·21	4·4	87
Gurdaspur	13th Dec. 1933	68·6	9·51	0·56	10·07	11·72	0·34	7·76	5·9	81
	25th Feb. 1934	67·5	12·34	0·14	12·48	13·72	0·41	10·76	1·1	90
Rawalpindi	15th Dec. 1933	68·2	9·17	0·49	9·66	11·49	0·42	7·21	5·4	80
	22nd Feb. 1934	67·7	7·61	1·98	9·59	11·60	0·30	4·58	26·0	66

TABLE IX

Analytical data for Co. 300 from different localities in the Punjab

Locality	Date of analysis	Juice per cent	Sucrose per cent	Glucose per cent	Total sugar per cent	Total solid per cent	Ash per cent	Nett rendiment	Glucose ratio	Purity
Lyallpur	15th Dec. 1933	68·5	8·92	0·36	9·28	11·17	0·67	6·23	4·1	80
	21st Feb. 1934	67·6	9·30	0·32	9·62	11·94	0·63	6·77	3·5	77
Sargodha	16th Dec. 1933	67·7	9·89	0·38	10·27	11·49	0·48	8·18	3·9	86
	19th Feb. 1934	67·9	9·92	0·35	10·27	12·48	0·50	7·82	3·5	80
Montgomery	17th Feb. 1933	65·3	9·90	0·14	10·00	11·0	0·44	8·23	1·4	90
	18th Feb. 1934	62·1	10·00	0·14	10·14	11·8	0·58	7·83	1·4	85
Multan	Not available									
Hansi	15th Dec. 1933	63·2	9·9	0·22	10·10	11·4	spoiled		2·2	87
	23rd Feb. 1934	65·6	8·4	0·25	8·65	11·9	0·51	6·36	3·0	71
Jullundur	13th Dec. 1933	70·1	7·8	1·01	8·80	10·0	0·58	4·76	12·9	78
	19th Feb. 1934	69·2	11·8	0·15	11·95	13·6	0·57	9·65	1·3	87
Gurdaspur	13th Dec. 1933	67·1	9·91	0·45	10·36	11·67	0·27	8·51	4·5	85
	25th Feb. 1934	67·1	11·88	0·22	12·10	13·17	0·31	10·57	1·7	90
Rawalpindi	15th Dec. 1933	70·9	9·89	1·25	11·14	12·23	0·48	6·96	12·6	81
	22nd Feb. 1934	70·0	7·88	1·49	9·37	11·05	0·27	5·44	19·0	71

It will be seen that, as a general rule, the mineral matter in the juice of all the varieties is least in the sub-montane tracts and increases progressively through the central districts towards the south-western arid tracts, where it attains the maximum value. The central regions, represented by the district of Jullundur and part of Hoshiarpur, produce canes with a medium mineral

content. This, combined with the fact that canes in this locality give the greatest yield of sucrose per acre, and experience a very long crushing season, as has been already pointed out by the authors [1935], might have been one of the factors which inadvertently exercised an influence in making this tract the home of the sugar industry in the past.

Similar variations also occur in the ash content of the juice of sugarcane grown in different localities in the United Provinces [Table X*]. It is interesting to note that the canes from Muzaffarnagar which is nearer to the source of the Jumna and the Ganges than Cawnpore, should be characterised by a low ash content, while those from Cawnpore which is much further away should possess a much higher content of mineral matter in their juice. It is difficult to say whether this is merely a coincidence or whether it reflects in any way conditions similar to those found in the Punjab where it is noticed that as we move with the rivers in a south-westerly direction so does the amount of mineral matter in the cane juice tend to increase. It is clear, however, that the ash content of the canes from the United Provinces is lower than that from the Punjab canes and this appears to be the only factor which detracts from their quality.

TABLE X

Ash content of the juice from the same varieties of sugarcane from different stations in the United Provinces

(Percentage ash on juice by weight)

Variety	Muzaffarnagar	Shahjahanpur	Cawnpore
Co. 213	0·270	0·374	0·578
Co. 281	0·287	0·432	..
Co. 285	0·497	..	0·685
Co. 300	0·278	0·559	0·515
Co. 312	0·269	0·409	0·381
Co. 313	0·381	0·406	0·664

3. Composition of the soil and the ash content of the juice of sugarcane growing thereon

As all the mineral elements present in plants are derived from the soil, it is necessary to examine the soil for a possible explanation of the peculiar behaviour of the same varieties of sugarcane in removing different amounts of minerals from different localities in the province. During the first year of this

* We are indebted to the Cane Agronomist, Muzaffarnagar, the Economic Botanist, Shahjahanpur, and the Agricultural Chemist, Cawnpore, for the supply of sugarcane juice of these varieties from their respective stations.

enquiry thirty-two samples of soil, on which the canes analysed were grown, were obtained from Jullundur, Gurdaspur, Gujranwala and Lyallpur and analysed for water-soluble salts. As has been remarked already no correlation was found to exist between these salts and the amount of mineral matter in the juice of the sugarcane. Thus, for instance, of the four places under observation, the Gurdaspur soil contained the largest amount of water-soluble salts, yet the canes from this place contained the least amount of soluble mineral matter in the juice.

Table XI gives the mechanical composition of some of the Punjab soils from which different varieties of sugarcane were obtained in the second year of this enquiry. It also includes the analyses of some typical sugarcane soils from the United Provinces and Bihar, while Table XII shows the figures for different water soluble ingredients of the same soils.

TABLE XI

Mechanical analyses of sugarcane soils

(Average figures)

Percentage on air-dried soil

Locality	Clay	Silt	Fine sand	Sand	Gravel
Pusa	7·20	45·80	46·60	0·41	..
Muzaffarnagar	12·07	30·08	52·47	5·11	0·27
Shahjahanpur	8·18	19·37	67·59	3·18	1·68
Gorakhpur	11·14	33·99	45·31	9·44	0·12
Hansi	15·80	26·10	57·83	0·24	0·02
Montgomery	12·45	31·66	54·66	0·98	0·25
Sirsa	6·70	13·40	79·03	0·78	..
Multan	13·88	31·94	53·82	0·31	0·06
Sargodha	11·42	30·40	45·48	12·60	0·10
Rawalpindi	21·32	34·71	41·55	2·16	0·26
Gurdaspur	10·84	28·90	38·58	21·55	0·13

It will be seen that, apart from the fact that the soils from the United Provinces and Bihar are slightly lighter than those from the Punjab, the soils from the three provinces do not reveal any striking differences in their mechanical constitution and the nature and composition of their water-soluble salts. Soils from Muzaffarnagar and Montgomery, as also from Gorakhpur and Sargodha are practically identical as far as their mechanical composition is concerned, but Sargodha and Montgomery soils do not produce as good a cane as do those from the other two places. Similarly, since soils from the United Provinces and Bihar contain less water-soluble salts than those from the arid regions (Multan and Montgomery) of the Punjab, a possible explanation of our problem may be found in this direction. It will be seen, however, that the Gorakhpur and

Sargodha soils, although they contain the same amount of water-soluble salts are not equally suitable as far as the quality of cane they produce is concerned. Again, Rawalpindi and Sargodha soils are identical in respect of the amount of water-soluble salts which they contain, but the former produces cane with the lowest amount of mineral matter in its juice of any in the Punjab. Thus it seems that differences in the mechanical composition of the soils and the salt content of their water extracts, do not provide a satisfactory explanation of the differences in the amount of mineral matter in the juices of cane obtained from different places.

TABLE XII

Analysis of sugarcane soils (average figures)

Ten per cent soil extract in water

Locality	Total solids	Carbonates	Bicarbonates	Chlorides	Sulphate
Pusa	0·155	0·010	0·103	0·029	..
Muzaffarnagar . . .	0·089	0·002	0·045	0·290	..
Shahjahanpur . . .	0·092	0·004	0·072	0·012	..
Gorakhpur . . .	0·126	..	0·068	0·025	..
Hansi	0·091	..	0·074	0·018	..
Sirsa	0·171	..	0·116	0·023	traces
Montgomery . . .	0·161	0·003	0·091	0·025	traces
Multan	0·179	0·003	0·085	0·035	0·075
Sargodha	0·131	..	0·095	0·012	..
Rawalpindi . . .	0·121	..	0·083	0·018	..
Gurdaspur . . .	0·080	..	0·051	0·014	traces

The analytical figures for hydrochloric acid-soluble salts for some of the typical soils from the above list are given in Table XIII. These indicate that, of the soils examined, those from Multan contain the highest amount of iron and aluminium. It is very difficult to gauge the extent to which differences in the amount of these two elements influence the quality of cane. It is worthy of note that the Pusa soils contain about 20 per cent of calcium, but only 0·158 per cent is present in the exchangeable form, recognised to be the more reactive state in which this element exists in the soil.

TABLE XIII

Chemical analyses of some typical sugarcane soil (hydrochloric acid extract)

Expressed as percentage on air-dried soil

Serial No.	Reg. No.	Description	Insoluble residue	Iron as Fe_2O_3	Aluminium as Al_2O_3	Calcium as CaO	Magnesium as MgO	Sodium and potassium as oxides	Phosphates as P_2O_5
1	364/34	Gurdaspur .	86·95	3·73	4·68	0·73	1·48	1·03	0·11
2	366/34	Gurdaspur .	87·64	3·50	4·86	0·63	1·10	0·91	0·10
3	221/34	Multan .	82·00	4·24	7·20	2·50	1·07	1·36	0·24
4	222/34	Multan .	80·54	4·78	7·46	2·45	1·14	1·47	0·17
5	12/34	Gorakhpur .	90·51	1·88	5·70	0·61	0·53	0·85	0·09
6	995/33	Pusa . .	54·50	1·45	4·16	21·00	1·42	1·00	0·20
7	997/33	Pusa . .	57·42	2·07	3·20	18·48	1·30	0·98	0·10

The exchangeable monovalent and divalent bases in these soils and the corresponding *p*H values are given in Tables XIV and XV.

The data in this table throw considerable light on the problem in hand, as it will be seen that the *p*H values of the soils from the arid regions are as a rule higher than those of the soils from the sub-montane, central or south-eastern regions.

It appears that soils with a *p*H value of 7 or less are best suited for the production of the highest quality juice, but this conclusion, which is based on the available data relating to the Punjab does not appear to be applicable to the United Provinces and Bihar soils. For example, the soils from Muzaffarnagar and Shahjahanpur have a *p*H value of 7, but those from Gorakhpur, Mushari and Pusa possess values approximating to those of soils from Sargodha and Lyallpur. This indicates that, while sugarcane does best on a soil with a *p*H value of approximately 7, it will tolerate those with values up to 7·7 and that the *p*H value of the soil alone is not necessarily an index of the quality of cane which it will produce. However, a better correlation seems to exist between the exchangeable calcium in the soil solution and the ash content of the juice.

The figures for the exchangeable calcium in the arid regions of the Punjab are very low, never being greater than 0·15 per cent on the soil. On the other hand, the soils from the United Provinces, Bihar and the Gurdaspur and Rawalpindi Districts of the Punjab, which produce canes with a low mineral content, show an exchangeable calcium figure lying between 0·15 and 0·20. The soil sample from Hansi, however, possessed the greatest amount of exchangeable calcium, namely 0·248, yet correspondingly the canes from Hansi did not show the lowest amount of mineral matter in their juice. With this single exception, a high content of exchangeable calcium in the soil seems to run *pari pasu* with low mineral figures in the juice. It is difficult to say in what precise way the exchangeable calcium in the soil affects this qualitative feature of the juice, but one obvious effect would be to render the soil better drained by improving its permeability to water.

TABLE XIV

Exchangeable bases in sugarcane soils

Serial No.	Reg. No	Description of the sample	*p*H value	Exchangeable K_2O	Exchangeable Na_2O	Exchangeable CaO	Exchangeable MgO	Ratio of monovalents to divalents
1	362/1934 .	Rawalpindi Agri. Farm . 1st foot	6·88	0·009	0·011	0·204	0·022	11·30
2	363/1934 .	Sargodha Agri. Farm . ,, .	7·56	0·096	0·008	0·152	0·022	1·67
3	364/1934 .	Gurdaspur Agri. Farm . ,, .	6·74	0·015	0·013	0·181	0·084	9·48
4	365/1934 .	Ditto . ,, .	6·78	0·005	0·017	0·174	0·049	10·12
5	366/1934 .	Ditto . ,, .	6·78	0·004	0·052	0·150	0·048	3·54
6	367/1934 .	Ditto . ,, .	6·91	0·007	0·054	0·159	0·051	3·46
7	106 . .	Hansi Agri. Farm . . ,, .	6·83	0·011	0·048	0·258	0·040	5·05
8	107 . .	Ditto . ,, .	7·24	0·006	0·044	0·234	0·032	5·32
9	108 . .	Ditto . ,, .	7·05	0·0045	0·058	0·252	0·044	4·70
10	109 . .	Sirsa Agri. Farm . . ,, .	7·38	0·005	0·034	0·136	0·015	3·86
11	212 . .	Montgomery Agri. Station ,, .	7·58	0·022	0·022	0·123	0·039	3·69
12	213 . .	Ditto . 2nd foot	7·90	0·024	0·008	0·112	0·036	4·63
13	214 . .	Ditto . 3rd foot	7·58	0·005	0·005	0·106	0·023	12·90
14	215 . .	Ditto . 1st foot	8·10	0·052	0·032	0·126	0·040	1·96
15	216 . .	Ditto . 2nd foot	8·08	0·029	0·008	0·083	0·024	2·89
16	217 . .	Ditto . 3rd foot	8·18	0·016	*Nil*	0·061	0·028	5·57
17	218 . .	Multan Agri. Farm . . 1st foot	8·08	0·032	0·005	0·094	0·016	2·97
18	219 . .	Ditto . 2nd foot	7·81	0·025	0·005	0·082	0·012	3·13

TABLE XIV—*contd.*

Serial No.	Reg. No.	Description of the sample	*p*H value	Exchangeable K_2O	Exchangeable Na_2O	Exchangeable CaO	Exchangeable MgO	Ratio of monovalents to divalents
19	220 . .	Multan Agri. Farm . 3rd foot	7·71	0·029	0·012	0·142	0·016	3·85
20	221 . .	Ditto . 1st foot	8·08	0·044	0·022	0·112	0·016	1·94
21	222 . .	Ditto . 2nd foot	7·95	0·041	0.082	0.112	0·012	1·00
22	223 . .	Ditto . 3rd foot	7·81	0·002	0·035	0·115	0·018	3·33
23	6 . .	Village Surju, Dist. Muzaffarnagar .	6·83	0·005	0·003	0·166	0·020	23·25
24	7 . .	Village Kukra, Dist. Muzaffarnagar .	7·08	0·042	0·041	0·162	0·024	2·24
25	8 . .	Village Musa, Dist. Muzaffarnagar .	6·83	0·015	0·009	0·179	0·024	8·45
26	9 . .	Narainpur, Dist. Shahjahanpur . .	7·11	0·017	0·011	0·197	0·016	7·60
27	10 . .	Banwaripur, Dist. Shahjahanpur .	7·19	0·022	0·019	0·199	0·032	5·63
28	11 . .	Atsillia, Dist. Shahjahanpur . .	..	0·019	0·019	0·200	0·008	5·47
29	12 . .	Maharajgunj, Dist. Gorakhpur . .	6·88	0·018	0·005	0·156	0·020	7·67
30	13 . .	Dharudumat, Dist. Gorakhpur . .	7·71	0·021	0·014	0·218	*Nil*	6·23
31	14 . .	Padvanna, Dist. Gorakhpur . .	8·01	0·026	0·008	0·174	0·028	5·94
32	995/1933 .	Mushari Sugar Research Station .	7·93	0·042	0·023	0·119	0·032	2·33
33	996/1933 .	Ditto . . .	7·45	0·026	0·046	0·187	0·016	2·82
34	997/1933 .	Pusa Research Institute . . .	7·68	0·055	0·006	0·156	*Nil*	2·57
35	998/1933 .	Ditto . . .	7·63	0·012	0·012	0·159	0·005	6·83
36	..	Lyallpur Normal Soil Sq. 27 . .	7·40	0·0268	0·029	0·140	0·014	2·75

TABLE XV

Exchangeable bases in sugarcane soils (average figures)

Serial No.	Description	*p*H value	Exchangeable K_2O	Exchangeable Na_2O	Exchangeable CaO	Exchangeable MgO
1	Rawalpindi .	6·88	0·009	0·001	0·204	0·022
2	Sargodha . .	7·56	0·096	0·008	0·152	0·022
3	Gurdaspur . .	6·80	0·008	0·034	0·167	0·058
4	Hansi . .	7·04	0·007	0·050	0·248	0·039
5	Sirsa . . .	7·38	0·005	0·034	0·136	..
6	Montgomery .	7·90	0·049	0·015	0·102	0·031
7	Multan . .	7·91	0·029	0·027	0·110	·010
8	Muzaffargarh .	6·94	0·021	0·018	0·169	..
9	Shahjahanpur .	7·16	0·019	0·016	0·179	0·019
10	Gorakhpur . .	7·53	0·022	0·009	0·183	·021
11	Mushari . .	7·69	0·034	0·035	0·153	0·024
12	Pusa . . .	7·66	0·035	0·009	0·158	·005
13	Lyallpur . .	7·60	0·027	0·029	0·140	0·014

Table XVI shows the relative permeability of some of these soils to water and it appears that the intake of salts by the cane is greatly influenced by the permeability of the soil on which it grows.

TABLE XVI

Showing relative permeability to water of different soil samples

Locality from which soils were obtained	C.c.s of water percolating through a 18·5 cm. column of soil in 4 hours	Exchangeable calcium
Shahjahanpur (Narainpur)	67·0	0·197
,, (Banwaripur)	29·0	0·199
Gorakhpur (Maharajgunj)	38·0	0·156
,, (Dharudumat)	18·6	0·218
Gurdaspur (Agricultural Station) . . .	31·6	0·181
,, ,, . . .	24·5	0·174
Muzaffarnagar (Surja)	8·4	0·166
,, (Kukra)	9·6	0·162
Mushari (Sugarcane Station, Bihar) . . .	8·4	0·119
Pusa (Imperial Agricultural Institute Farm) .	9·5	0·156
Lyallpur (Agricultural Farm)	7·9	0·140
Montgomery ,, ...	6·4	0·123
,, ,, ...	8·8	0·126
Multan ,, ...	2·1	0·094
,, ,, ...	6·2	0·112

4. Possibilities of controlling the juice-soluble mineral matter in the sugarcane crop in the Punjab

If there is an abundance of soluble inorganic salts present in the soil, most plants will absorb a greater quantity than is necessary for their maximum growth. Thus, Jordon [1913] in his nutrition experiments with barley, peas, tomatoes, tobacco, buckwheat and rape found that both phosphorus and potash compounds increased in these plants after the maximum increase in growth had been reached. As already noted, however, we did not find any direct relationship between the amount of soluble salts present in the soil and the mineral content of the sugarcane juice. The above observation of Jordon, however, refers not to the expressed sap but the whole plant. Further, it has been noted that the amount of a given element in a plant may be influenced by the proportion and kind of other elements contained in the soil or culture solution. Thus Ginsburg [1925] found that withholding phosphorus increased the ash content of the soybean plant from 13·3 per cent to 18·6 per cent and similarly withholding manganese increased it to 18·4. The figures (Table XVII) obtained at Lyallpur indicate the possibility of modifying the composition of the plant by changing the proportions of different elements in the soil, and in view of the findings of Lipmann [1904] that the nature of the salts present in the juice exercise a profound effect in determining the amount of sucrose withheld from crystallising (as shown below) the above observations are of considerable importance. Lipmann refers to Lagrange who showed that when present in pure sugar solution, one part of the following salts will prevent sugar from crystallising in amounts shown against each :—

	Amount of sugar prevented from crystallising out
	Parts
NaCl	0·0
CaCl	0·5
Na_2SO_4	2·0
KCl	3·0
K_2SO_4	3·5
Na_2CO_3	3·5
K_2CO_3	3·5
Na_3PO_4	5·0
KNO_3	5·5
$NaNO_3$	6·5

TABLE XVII

Mineral composition of wheat plant grown on soils saturated with different cations (metals)

Nature of cation	Ash	Insoluble residue	Iron and alumi-nium	Calcium	Magne-sium	Manga-nese	Sodium	Potas-sium
Calcium . .	17·95	5·82	1·218	1·424	0·698	0·010	0·855	3·87
Magnesium .	15·74	4·16	0·785	0·057	1·192	0·010	1·380	4·87
Manganese . .	10·04	2·63	0·579	0·746	0·522	1·215	0·288	2·49
Potassium . .	20·40	4·47	0·639	0·590	0·530	0·012	0·796	9·41
Normal soil .	11·41	...	...	0·470	0·310	0·012	0·710	2·97

Lipmann refers in detail to the investigations of a number of workers such as Marschal, Williamson, Nugues, Herzfeld, and others, on the determination of the molasses forming power of different salts and draws the general conclusion that the recovery of white sugar from sugarcane juice is influenced not only by the amount of mineral matter but also by the nature of the salts present in the juice. It has further been seen that the nature and amount of salts taken up by plants depend upon the composition of the soil or culture medium in which they grow. These observations suggest the possibility of decreasing the amount of mineral matter in cane juice (particularly in regard to certain ingredients) by treatment of the soil on which the cane grows. As has already been pointed out soils with a high exchangeable calcium content have yielded canes with a low ash content.

It appears, therefore, that it should be possible in the Punjab to produce canes with the lower ash content than at present by increasing the exchangeable calcium in the soil by a judicious application of calcium salts. It has already been found at Montgomery that the juice of sugarcane (Co. 285) from soil treated with gypsum gives 0·98 gr. of ash per 100 c.c. against 1·12 from an untreated soil. Further, Hurst and McKaig [1933] found that the application of nitrogen fertilisers increased the nitrogen content of cane juice to an appreciable extent and also decreased the ash content of the juice as compared with juice from cane grown on unmanured soil. On the other hand they found that the potassic or phosphatic fertilisers increased the ash content of the juice. In order to throw further light on these features, a series of experiments under controlled conditions, using calcium and nitrogen fertilisers, have been started at Multan, Lyallpur and Gurdaspur, and the results will be presented when available.

SUMMARY

1. Sugar refineries in the Punjab have always shown a preference for *gur* or jaggery from the United Provinces over the local commodity, on the plea that the former yields a greater percentage of white sugar. It was at one time believed

that the smaller recovery of cane sugar from Punjab *gurs* was due to the defective method of their preparation, resulting in the breaking down into smaller fragments of the larger-sized crystals of sucrose by constant ladling and stirring of the juice and the thickened syrup as practised by the Punjabi *gur* boiler—an obviously unacceptable explanation.

2. It has, however, been found that the low recovery is due to the greater amount of mineral matter present in the Punjab *gurs*, which is the result of the high ash content of the cane-juice from which these are prepared.

3. Apart from the higher ash content of their juice, the Punjab canes are in no way inferior to those from the United Provinces and Bihar ; they contain as much sucrose as those from the two latter provinces and much less glucose.

4. Since all mineral matter in plants and crops is derived from the soil on which they grow, the high mineral content of the juice from the Punjab canes must be correlated with the composition of the Punjab soils. It has been shown that in mechanical composition and the nature and amount of their water- and acid-soluble salt content, the sugarcane soils of the Punjab are almost identical with those from the other two provinces, but they differ in the amount of exchangeable calcium. This in conjunction with a slightly higher *p*H value seems to be the most likely explanation of the greater ash content of the juice from sugarcane grown in the Punjab.

5. It is suggested on the analogy of the wheat plant that it may be possible to modify the ash content of the cane juice by treatment of the soil with certain chemicals and manures. Experiments with this object in view are in progress at different centres in the province and the result will be communicated when ready.

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THE PROBLEM OF THE NITROGEN SUPPLY OF RICE

I. FIXATION OF NITROGEN IN THE RICE SOILS UNDER WATER-LOGGED CONDITIONS

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An excerpt from Crop Production in India by Albert Howard is given below to introduce the subject under investigation. Referring to the phenomenon of nitrogen-supply of rice, Howard states:

"In any scientific consideration of the soils which produce the rice crop in India, practical experience at first seems to contradict one of the great principles of the agricultural science of the Occident, namely, the dependence of cereals on nitrogenous manures. Large rice crops are produced in many parts of India on the same land year after year, without the addition of any manure whatever. The rice fields of the country export paddy in large quantities to the centres of population or abroad, and there is no corresponding import of combined nitrogen. Taking Burma as an example of an area exporting rice beyond seas, during the last twenty years about 25,000,000 tons of paddy have been exported from a tract roughly 10,000,000 acres in area. As unhusked rice contains about 1·2 per cent. of nitrogen, the amount of this element shipped overseas during the last twenty years or destroyed by the burning of the husk is in the neighbourhood of 300,000 tons. As this constant drain of nitrogen is not made up by the import of manure, we should expect to find a gradual loss of fertility. Nevertheless this does not take place either in Burma or in Bengal, where rice has been grown on the same land year after year for centuries. Clearly the soil must obtain supplies of nitrogen from somewhere, otherwise the crop would cease to grow. The only likely source is the fixation from the atmosphere and the question arises how can this take place in a Bengal rice field, for example."

There are obviously two possibilities to account for the ability of rice to grow in the same soils year after year even in absence of any manure. Either sufficient nitrogen is fixed from the atmosphere and rendered available in the soil to compensate for the amount removed with the crop and lost otherwise or the plants themselves are able to assimilate elementary nitrogen like legumes. Although in the present state of our knowledge an assumption that a cereal crop like rice can fix atmospheric nitrogen will hardly receive any support, nevertheless this possibility deserves very careful examination in view of the fact that Sen [1929] has demonstrated the occurrence of a nitrogen-fixing organism within rice root while Viswanath [1932] obtained indications that rice plant may possess the power of fixing nitrogen.

In studying the fixation of nitrogen in the rice soils, it should be remembered that these soils remain alternately dry and water-logged. From transplantation to harvest, soils remain water-logged : thereafter up to the next transplantation they are allowed to remain dry and fallow. In the water-logged state the conditions become distinctly unfavourable to the beneficial flora of the soil such as *Azotobacter*, and it is, therefore not expected that any bacterial fixation of nitrogen will take place at this period. But the abundant growth of the algal film very often observed on the surface of the submerged rice soils, deserves very serious consideration. The beneficial effect of these organisms in aerating the roots of the rice plant and the upper layers of the submerged soils have been observed by Harrison and Aiyer [1914]. Nothing, however, is known whether they are able to fix nitrogen, though Howard [1924] suggested that ' the algae and bacteria in the film, working conjointly, are capable of using the energy of sunlight to fix some of the nitrogen evolved in fermentation in a form suitable to the rice plant '. Several investigators have described a symbiosis with the nitrogen-fixing bacteria on the part of algae ; others have affirmed in a belief that algae themselves are able to fix (direct) nitrogen. For earlier literature on this subject, reference may be made to a paper by Bristol and Page [1923] whose very careful experiment has shown that green algae in pure cultures cannot fix elementary nitrogen. Amongst the recent workers, Lipmann and Teakle [1925] observed that an association between *Azotobacter* and *Chlorella* led to an increased fixation of nitrogen though Hoover and Allison [1935] claim that certain *Nostoc* are able to fix nitrogen in pure cultures.

Some fixation is also possible during the dry period, particularly in the winter when the conditions of the soils, as regards temperature and moisture, are eminently suitable for the growth of *Azotobacter* and other nitrogen-fixing bacteria. The average soil-temperature at this stage is about 25°C. and the moisture-content is sufficiently low to secure aerobic conditions. Moreover, a portion of the straw is usually left in the fields after harvest which may be utilised by the nitrogen-fixing bacteria as source of energy.

Besides the fixation from the atmosphere, soils may receive nitrogen from other sources as well. With dew, rains, etc., a small amount of nitrogen may be added. Leather [1906] calculated that in North India about 3·2 lbs. of nitrogen are added per acre of land in this way. Deposition of silts is another important factor but the supply from this source is restricted only to the areas irrigated with river water.

The investigations outlined above were started in this laboratory in 1931 and the results obtained so far will be described in this series of papers.

Experimental

The first experiment of the series was performed in 1932 with a few samples of soils collected from different plots of the Government Farm at Dacca. Fifteen

grms. portions of air-dried and sieved soils were water-logged with the addition of 100 c.c. of distilled water in 300 c.c. Erlenmeyer flasks. The flasks were divided into two sets—one set was exposed to sunlight and the other was kept in the dark. Determination of total nitrogen after three months showed that the soils kept in the light gained slightly in nitrogen while there was significant loss from those in the dark. The experiment was repeated several times and the previous observations confirmed. The results of one of the experiments are given in Table I.

Very encouraging results were obtained when similar experiments were performed with a sample of soil obtained from the district of Faridpur. In this soil a greenish algal growth appeared soon after water-logging and exposure to sun, which gradually increased in bulk and eventually became so heavy that the entire surface of the soil was covered with a thick growth of it. Determination of total nitrogen after three months showed considerable gain. As in Dacca soil, slight loss of nitrogen took place in the dark.

TABLE I

Showing the fixation of nitrogen in Dacca and in Faridpur soils under water-logged conditions

(Nitrogen as p. p. m.)

Soil	*N* at start	*N* after exposure to sunlight
DACCA.		
Plot North Hazi . . .	900	952, 924, 933, 925.
Plot East Ravina . . .	1312	1344, 1325, 1325.
Faridpur	639	887, 887, 802.

Considering the importance of the results obtained with Faridpur soil in their bearings on the present problem, it was proposed to repeat the above experiment with a large number of replicates in order to confirm the previous observations. Accordingly the experiment was repeated in 1933 and again in 1934. The results of the latter experiment are given in the following table.

TABLE II

Showing the fixation of nitrogen in Faridpur soil

N as p. p. m.

N at start	N after exposure
635	784, 829, 802, 847, 856, 847, 865, 811.

In order to observe the influence of temperature and other environmental conditions on the fixation of nitrogen, the above experiment was performed both in the summer and in the winter but no appreciable differences were observed in the amounts of nitrogen fixed under the two conditions.

Attempts were next made to account for the low nitrogen fixing capacity of Dacca soil. It will be noticed that there was a heavy growth of algae in Faridpur soil exposed to light in which considerable fixation of nitrogen also took place. On the other hand the growth in the corresponding Dacca soil was very poor and absolutely no growth appeared in any of the soils kept in the dark. And in these cases, little or no fixation of nitrogen took place. It was, therefore, thought that these algae play an important role in the fixation of nitrogen and their failure to develop in Dacca soil is the cause of its low nitrogen-fixing capacity. Two reasons may be suggested to account why the growth was so poor in Dacca soil : the organisms themselves may not be present in the soil in an active condition or the conditions of the soil might not be favourable for their growth. If former is the cause, there should be an increased fixation of nitrogen in the soil if it is inoculated with the appropriate organisms. But if the latter, no such increase will take place. This was tested by water-logging and exposing to sunlight 15 grms. portions of soil (14 grms. of Dacca soil + 1 grm. of Faridpur soil) and determining total nitrogen at the end of the experiment. The amounts fixed under these conditions were not sensibly different from those fixed in Dacca soil alone. Obviously the conditions obtaining in Dacca soil were not favourable for the growth of the organisms supplied with Faridpur soil. On comparing the properties of these two soils, an important difference was found in their reaction. Whereas, Faridpur soil is slightly alkaline (pH 8·2), Dacca soil is acidic (pH 4·9). Suspecting that the low pH of Dacca soil was the factor inhibiting the growth of the organisms responsible for the fixation of nitrogen, 15 grms. portions of the soil were water-logged after addition of 1 grm. of $CaCO_3$ and the mixtures exposed to sunlight. Soon a growth similar to that observed in Faridpur soil appeared in the cultures and the determination of nitrogen showed considerable increase (Table III). It is, therefore, clear that the organisms were already present in the soil but they could not grow owing to the acid reaction of the soil.

TABLE III

Showing the fixation of nitrogen in Dacca soil after treatment with $CaCO_3$

Nitrogen as p. p. m.

——	N at start	N after exposure to sunlight
DACCA SOIL		
Plot North Hazi . . .	821	1073, 1073, 1017, 989, 1026.
Plot East Ravina . . .	1296	1428, 1530*, 1700*, 1578*, 1587* 1730*.

* Exposed to sunlight for 7 months ; the remaining cultures were exposed for 3 months.

Next, in order to ascertain whether the fixation of nitrogen in the water-logged state is a special property possessed only by Dacca and Faridpur soils or by the rice soils in general, a number of rice soils were collected from different parts of India and the fixation of nitrogen in them was studied, the procedure being identical with that described before. The result are given in Table IV.

TABLE IV

Showing the fixation of nitrogen in different soils

Nitrogen as p. p. m. on air-dry basis

Soil	*p*H	N at start	N after exposure	Gain
Rajshahi, Bengal	8·20	1069	1402	333
Bhagalpur, Bihar	8·45	940	1270	330
Coimbatore, Madras	8·13	789	1064	275
Raipur, C. P. Dorsa	7·76	849	961	112
Raipur, C. P. Matasi	7·63	448	546	98
Karjat, Bombay	7·66	588	723	135
Rangpur, Bengal	6·20	1036	1185	149
Insein, Burma	5·10	562	641	79
Sylhet, Assam (B)	4·95	1097	1138	41
Sylhet, Assam (C)	4·98	1566	1577	11

In the above experiment, total nitrogen at start, was determined both by Kjeldahl method modified to include nitrate and by the ' wet digestion ' method of Srinivasan [1932].

The results in Table IV show that considerable amount of nitrogen has been fixed in the soils having slightly alkaline reaction. In these soils, very heavy growths, similar to that observed in Faridpur soil, appeared soon after water-logging. In some cases, the growths were so thick that the entire soil mass remained embedded within them. On the other hand, little or no growth was observed in soils with lower *p*H, though on the addition of lime, even these soils showed good growths. It appears very probable that the growths appearing in the different soils are not alike. There is considerable difference in the shades of their colour. Growth from Dacca soil was nearly colourless, from Coimbatore soil brown, from Faridpur soil light green while those appearing in Rajshahi and Bhagalpur soils were deep green.

It was next intended to see whether on treatment with lime the nitrogen-fixing capacities of the soils specially of those having low *p*H could be improved as has been done in the case of Dacca soil. For this purpose 15 grms. portions of each of the following soils were mixed with 1 grm. of $CaCO_3$ and the mixtures water-logged in the usual way. In every case, the growths were much better than in the untreated soils. The results (Table V) show the beneficial effect of lime on the fixation of nitrogen in water-logged soils.

TABLE V

Showing the effect of lime on the fixation of nitrogen. Nitrogen as p. p. m. on air-dry basis.

Soil	N fixed	
	without lime	with lime
Sylhet, Assam (B)	41	116
Sylhet, Assam (C)	11	142
Rangpur, Bengal	149	205
Insein, Burma	79	138
Karjat, Bombay	135	308

ON THE NATURE OF THE ORGANISMS RESPONSIBLE FOR THE FIXATION OF NITROGEN IN WATER-LOGGED SOILS

From the observations made with Dacca and Faridpur soils, it is evident that the fixation of nitrogen in water-logged soils is not a bacterial process. For, if it were bacterial, not only fixation would have taken place in the dark but perhaps greater amount would have been fixed under this condition than in the sunlight on account of the germicidal property of the latter. The following experiment shows that bacteria actually fix more nitrogen in the dark than in the light.

A number of Erlenmeyer flasks each containing 100 c.c. of Ashby's solution were each inoculated with 1 grm. of Faridpur soil. For experiment in the dark, the flasks were painted outside by a thick coating of Japan Black and covered with small beakers similarly painted. The flasks were exposed to sunlight for two weeks after which total nitrogen was determined. The results are given in the following table.

TABLE VI

Showing the influence of sunlight on the fixation of nitrogen by bacteria

N as mgrm. fixed per 100 c.c. of Ashby's solution

Dark	Light
9·1	6·0
8·5	5·7
6·7	6·4
8·1	8·5
10·1	8·5
Mean 8·5	7·1

It was next thought that the algae that appeared in the soils on water-logging, are the active agents. To ascertain whether these algae actually possess the power of nitrogen-fixation, a little growth from Faridpur soil was inoculated into a medium containing the necessary minerals but no combined carbon or nitrogen and the solution exposed to sunlight. Within a week, a greenish growth was appearing at first on the inner bottom of the flasks but gradually rising up to the surface of the solution. At the end of two months scattered growths, both floating and submerged, were seen throughout the solution. The growths were slimy, pale green in colour and had large bubbles of gases enclosed within their mass. Determination of total nitrogen showed an average fixation of 3·6, mgrm. of nitrogen per 100 c. c. of the culture solution. From this experiment, as well as, from the observations that considerable amount of nitrogen was fixed in those cases where algal growth was abundant, it is concluded that the fixation of nitrogen in water-logged soils is an algal process. But as the above experiment was performed with crude cultures, it is not possible to say at present whether the fixation is brought about by algae themselves or by algae working in symbiosis with bacteria. The first step in the settlement of this question is obviously to obtain the different species of algae in a state completely free from bacteria. The individual species may then be tested for their nitrogen-fixing power. Attempts were accordingly made to purify the algal cultures by successively sub-culturing them in a medium free from combined carbon and nitrogen. The composition of the medium is given below. As this medium is not suitable for the growth of bacteria and other non-nitrogen-fixing organisms, it was thought that they would be eliminated after a few sub-cultures.

K_2HPO_4	0·5 grm.
$MgSO_4, 7H_2O$	0·2 grm.
$CaSO_4$	0·1 grm.
$FePO_4$	0·1 grm.
$Ca_3(PO_4)_2$	1·0 grm.
$FeCl_3$	Trace.
H_2O (glass distilled)	1000 c.c.

Sub-cultures were made by transferring asceptically one drop of a thoroughly shaken suspension of the growth from the previous culture to 100 c. c. portions of sterilised medium. The amounts of nitrogen fixed by different sub-cultures are shown in the following table.

TABLE VII

Showing the amounts of nitrogen fixed by different sub-cultures

	N as mgrm. fixed per 100 c. c. of the medium
First subculture	3·6
Second „	2·6
Third „	7·6*
Fourth „	2·2
Fifth „	2·1

Several of the above cultures were kindly examined by Professor F. E. Fritsch, F.R.S., of the Queen Mary College, London. The following extract from his report will show the conditions of the cultures after purification. Referring to the fifth sub-cultures, Professor Fritsch says:

"Tube No. 1 contains a blue-green alga, together with what I take to be a number of fungal hyphæ and a good deal of an undeterminable green unicellular alga. The blue-green alga, which makes up the bulk of the sample, is a species of *Phormidium* closely related to *P. orientale* West. It differs from the published descriptions in narrower width, shorter cells, and the pale colour of the trichomes. Very occasional threads, however, have the elongate cells of the type. I should be inclined to suspect that the shortness of the cells might be a result of rapid cell-division under cultural conditions and would, therefore, regard the material as a cultural form of *Phormidium orientale* showing the differences above enumerated."

He has further reported that 'several of your cultures also contain Bacteria and perhaps Fungi'.

Consequently it is not possible to say at this moment whether the fixation of nitrogen in water-logged soils is the result of the activities of algae alone or of algae working in symbiosis with either bacteria or fungi. The author hopes to carry out this work further in the laboratory of Professor Fritsch and the results that will be obtained there will be published in a separate paper.

SUMMARY

(1) Several rice-soils collected from different parts of India were studied with regard to their power to fix nitrogen under water-logged conditions.

(2) Fixation of nitrogen took place in all of the soils examined, when they were water-logged and exposed to sunlight for three months.

(3) Soils having slightly alkaline reaction fixed much more nitrogen than those with lower *p*H. In the former soils, a heavy algal growth appeared soon after water-logging but in the latter the growth was much poor.

(4) The addition of calcium carbonate to the soils having acid reactions stimulated both algal growth and also nitrogen fixation.

* Exposed to sunlight for 11 months; the rest were exposed for 2 months.

(5) It has been shown that bacteria fix more nitrogen in the dark than in presence of sunlight.

(6) From the evidences available, it is concluded that the fixation of nitrogen in water-logged soils is an algal process, though it has not yet been possible to decide whether the fixation is brought about by algae alone or in symbiosis with other organisms.

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INHERITANCE OF EARLINESS IN UNITED PROVINCES RICES, I

BY

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Introduction

The following account is the result of hybridization studies in earliness in crosses made in rice for achieving economic ends. Data have been accumulated up to the F_5 stage, and a few strains of promise have been extracted. All along the course of the work, earliness was one of the chief bases of selection, hence close study of the inheritance of earliness had to be pursued. The results are reproduced here because, except for two papers by Ramiah published in the previous numbers of this *Journal* [Ramiah, 1933], elaborate presentation of data on inheritance of earliness in rices in India is not easily available, due firstly to the paucity of presentable data, and secondly to their being scattered in different departmental reports and bulletins which fail to command the attention of workers in India and abroad. This work, which differs in certain respects from Ramiah's work, will, therefore, be of interest to workers in other parts of India and abroad.

Two kinds of crosses were made, *viz.*, (1) Between ordinary open-eared types, (Economic crosses) ; (2) Between open-eared types and enclosed-eared types (*Sathi* crosses).

In the present paper the results of only the former group are presented.

The economic crosses were made, in each case, between an early and a late-ripening type. What we mean by an early or a late-ripening type has already been

explained in an earlier publication [Sethi, Sethi and Mehta, 1936], which besides explaining this point, deals with the relation of flowering to ripening. It has not generally been pointed out that the ripening period (*i.e.*, the interval between flowering and ripening) varies in different varieties. Usually it has been stated that it is constant for all varieties. This is not true of the United Provinces rices.

Literature review

Earliness has been a subject of study in different crops by several workers. These studies have shown that earliness may be inherited in different ways even in the same crop. Usually it has been found that the F_1 is intermediate between the parents, its earliness often being near one or the other parent. The F_2 shows great variation and may or may not show grouping into definite classes. In F_3 and later generations, families with different flowering durations can be isolated, thus indicating definite segregation into different classes of earliness. To mention the work of some of the important workers, the name of Farrer [1898] may be mentioned first as being one of the first in the field. He found earliness to be intermediate between the parents in wheat. Biffen [1905] found earliness dominant in an interspecific cross between an early Polish and a late Rivet wheat. Earlier Mendel had found that the F_1 was intermediate in time of ripening in pea [Bateson, 1909]. Keeble and Pellew [1910] demonstrated a dominance of lateness over earliness in pea, although they found that the F_1 was intermediate and not late like the late parent. They conclude that " the time of flowering, though inherited, is modified in its expression in the zygote by morphological characters .... ". Leake [1911] found in cotton that the F_1 was intermediate between the parents, and the mean of the F_2, which was monomodal, was nearest the late parent. Emerson and East [1913] in a cross between a very early and a very late corn found that the cross produced an F_1 generation strictly intermediate between the parents in duration, F_2 generations with a range of variation from the early parent to, or nearly to, the late parent, and F_3 generations with diverse seasons of blossoming. Caporn [1918] found in an oat cross that the F_1 progeny was intermediate between the parents in ripening. The F_2 showed much variation but in F_3 the variation did not extend so far as that of the parents. Thompson [1918] studied numerous crosses in wheat and found the dominance of lateness in F_1. However, in F_2 the majority of individuals were intermediate, but the distribution was not broken up into groups. The apparent dominance of lateness in F_1 has been ascribed to hybrid vigour. Freeman [1918, 1919] demonstrated a partial dominance of lateness in wheat crosses. Florell [1924] in a Sunset-Marquis wheat cross concluded that earliness was dominant in F_2 giving 3·11 to 0·89 early and late plants, " indicating one allelomorphic pair of factors, with possibly a number of minor modifying factors ".

In rice one of the first reported studies in earliness is that carried on by van der Stok [1910]. He found earliness dominant in F_1 and a transgressive segregation

in F_2. Hoshino [1915] found F_1 early, F_2 divided into two groups, a large early group and a small late group. Of 340 F_3 families, five were constant for early habit and four for late habit, while the rest were segregating. On a tri-factorial basis the number of pure breeding families expected would be about ten, five early and five late, which is nearly what was obtained. Hence Hoshino advanced a tri-factorial interpretation to explain the results. Ikeno [1918] reporting the work done on rice by Dr. Kato stated that the F_1 was intermediate in earliness, and the F_2 showed a continuous segregation due to multiple factors. Hector [Evans, 1922] found that the F_2 hybrids of a cross between a high coloured autumn (early) rice and a colourless winter (late) rice segregated into two distinct periods (corresponding approximately to the two parents) separated by an interval of about three weeks during which no flowering took place. The ratio of early to late was about 3 : 1. Nomura and Yamazaki [1925] found the F_1 later than the late parent, and the F_2 showed a grouping into three late to one early plants. The late plants were on an average slightly later and the early plants slightly earlier than the late and early parents, respectively. The authors concluded that the inheritance in the crosses was controlled by three factors, **A**, **B**, and **C**, each of which prolonged the shooting time to a certain extent, **C** being most and **B** least efficacious in this respect. Bhide [1926] found lateness dominant, and in the F_2 of a cross and its reciprocal, the ratio of 2·84 and 2·75 late plants to 1 early respectively. Jones [1928] studied a number of crosses for earliness and found the F_1 behaving very differently in the different cases. It was either earlier than the early parent, or nearer the late parent, or as late as the late parent, or very much later than the late parent. In F_2 the population studied could not be classified with certainty into any one of the simple or modified Mendelian ratios. Transgressive segregation occurred, and the classification of the F_2 on the basis of fluctuation of the maximum temperature during the growth season into a small early and a large late group gave a 1 : 3 ratio. In the F_3 one family bred almost true for early maturity, and one almost true for late maturity. Three families segregated into a ratio of three lates to one early and one family produced plants in a distinct ratio one early to two mid-season to one late. Other families showed no definite segregation. Jones concludes that " the data presented indicate that two or more genetic factors are involved in the production of earliness in the rices studied ". Ramiah [1933] studied several crosses at Coimbatore and found that whereas the inheritance was quite simple in some cases showing a single factor difference between earliness and lateness, it was complicated in other cases and could be explained only under the multiple factor hypothesis. He found earliness generally dominant, but has recorded a single case where earliness was recessive.

Material studied

Seven economic crosses were studied. The results are almost alike, hence the description will be confined to only one cross and only the broadest outline of the results of other crosses will be given.

Cross No. 23 (T. 1 × T. 110)

Type 1 is a long fine-grained, early ripening variety possessing weak stem thereby showing a tendency to lodge.

Type 110 is a coarse round-grained, late ripening variety with stronger stem than T. 1. It is much too late in duration to become very popular with the cultivators. Both the types are among the selected types of the United Provinces rices.

The aim of the cross was to isolate—

(1) Early types, heavier in yield and better in stand than T. 1. T. 1, on account of its long grain breaks easily during hulling, and perhaps a slightly coarser grained type would get hulled better.

(2) Late types, with finer grain than the coarse grained T. 110, and with a slightly shorter duration.

The cross was made first in 1929, and later repeated in 1931. The flowering durations of the two types and the F_1 as observed in 1932 were as follows :

	Days
T. 1	89
T. 110	131
F_1	119

Thus, the parents differ from each other by forty-two days. The F_1 is intermediate but is very much nearer the late parent than the early one, indicating the dominance of lateness.

The F_2 and the parents grown in 1931 gave flowering durations stated in Table I.

TABLE I

Mean flowering durations in F_2 and parents in 1931

Culture	Number of individuals	Mean flowering duration	Coefficient of variation
T. 1	192	88·6	2·45
T. 110	259	132·0	0·54
F_2	865	116·8	10·88

The mean flowering duration of F_2 is again intermediate between the two parents, but very much nearer the late parent. The F_2 flowered in two distinct flushes, one early and other late. Table II gives the frequency distribution of the flowering durations, and Figure 1 gives the corresponding bimodal curve.

TABLE II

Frequency distribution of flowering duration in Types 1 and 110 and in F_2 in 1931

Culture	Flowering Duration																			Total
	82	85	88	91	94	97	100	103	106	109	112	115	118	121	124	127	130	133	136	139	
T. 1	3	22	93	64	10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	192
T. 110	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	76	183	...	...	259
F_2	5	19	15	38	32	35	24	11	1	1	1	27	128	218	115	114	55	18	4	4	865

The modes of the early and the late groups in F_2 do not coincide with the modes of the respective parents, but show a shift towards the middle, as is apparent from Fig. 1.

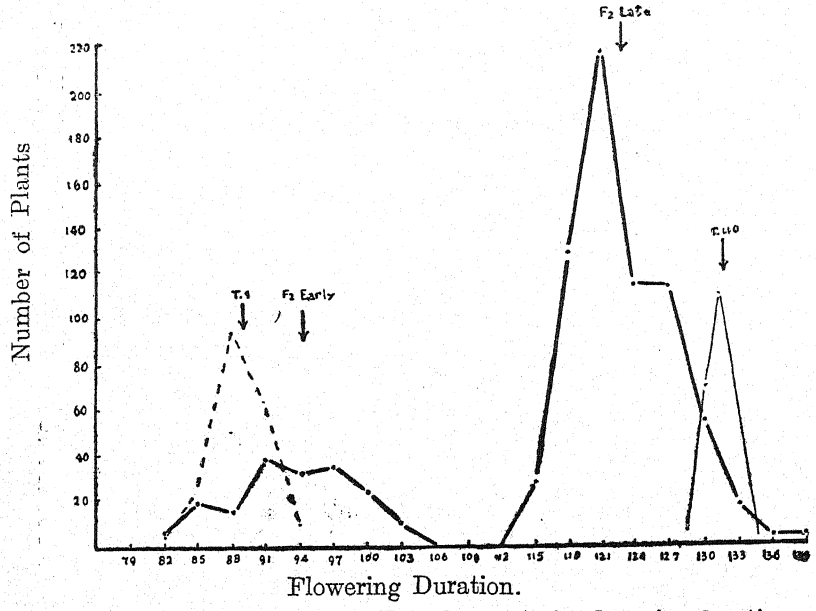

Fig. 1. Frequency distribution of F_2 and parents for flowering duration.

The early segregates hardly transgress beyond the parental limit on the early side, but a majority of them occupy a position later than the mean of the early parent. Similarly in the late segregates the transgression beyond the late extreme is very limited, but the majority of the segregates are earlier than the late parent. The various mean durations are indicated by arrows in Fig. 1 and are given along with their standard errors below.

T. 1	$88{\cdot}6 \pm 0{\cdot}1567$
T. 110	$132{\cdot}0 \pm 0{\cdot}0443$
Early segregates	$93{\cdot}8 \pm 0{\cdot}4166$
Late segregates	$122{\cdot}9 \pm 0.1816$

The difference between the mean duration of T. 1 and of the early F_2 segregates is 5·2 days, and the corresponding difference for the late parent and the late segregates is 9·1 days. These differences are statistically significant as indicated by the high values of $\frac{\text{difference in means.}}{\text{its S. E.}}$

T. 1 and early segregates:

$$\text{Thus ratio} = \frac{5\cdot2}{\sqrt{\cdot1567^2+0\cdot4166^2}} = 11\cdot68$$

T. 110 and late segregates:

$$\text{Thus ratio} = \frac{9\cdot1}{\sqrt{0\cdot0443^2+0\cdot1816^2}} = 48\cdot72$$

Thus there has been a definite 'shift' in the mean durations of the two phenotypes.

Table II as well as Fig. 1 show the dominance of lateness in the F_2. The frequencies are

	Early	Late	Total
Observed	182	683	865
Calculated on 1 : 3 basis	216·25	648·75	
Deviation	34·25		
$\frac{\text{Deviation}}{\text{Standard Error}}$	$\frac{34\cdot25}{12\cdot74}=2\cdot69$		

The frequencies suggest a 3:1 ratio of late and early plants, although the deviation from expectation is greater than would be caused by chance. However, the F_3 and the subsequent generations, as will be shown later, showed the split into a simple 3:1 ratio, and the deviations were always within the limits of random sampling.

F_3 Generation.—The F_3 generation consisted of 173 families in this cross. The dominance of lateness was confirmed in this generation. Out of the 173 F_2 parents 46 were from the early group and 127 from the late group. The F_3 families from the former all gave only early plants. Of the lates 67 families gave only late plants and 60 families segregated into earlies and lates. The totals from these segregating families and the corresponding figures expected on a 3 : 1 basis are as follows:

	Lates	Earlies	Total
Observed	5,164	1,650	6,814
Calculated	5,110·5	1,703·5	
Deviation	53·5		
S. E.	35·74		
$\frac{\text{Deviation}}{\text{S. E.}}$	=1·50		

The figures are within the range of expectation.

On a 3 : 1 basis it would be expected that out of the 127 late F_2 plants, about two-thirds (*i.e.*, eighty-five) would segregate in the F_3, and the one-third (*i.e.*, forty-two) would give only late plants. Actually the numbers of segregating and pure* late families are 60 and 67, respectively. This wide departure from the expected result will be referred to later and an explanation given.

The F_3 plants of each family were individually noted for flowering duration. The plants were properly numbered in the field with tags and the date of flowering of each plant was noted separately. The data of a few of the representative families of each of the three groups (*viz.*, pure early, pure late and the segregating groups) are summarised in Table III.

TABLE III

Segregation in some of the F_3 families of Cross T. 1 × T. 110 for flowering.

Nature of F_3 parents	Segregation in F_3	F_3 Family No.	F_2 Duration	F_3 Flowering			Segregating Families		Total No. of Plants	Percentage of early plants
				Range	Period	Mean Duration	Earlies	Lates		
Early .	Pure early	18	90	78—94	17	84	...	...	121	...
,,	,,	52	91	78—94	17	90	...	...	112	...
,,	,,	114	102	84—94	11	90	...	...	109	...
,,	,,	150	88	78—94	17	81	...	...	94	...
,,	,,	242	85	78—91	14	86	...	...	105	...
,,	,,	255	90	78—100	23	90	...	...	115	...
,,	,,	263	97	78—103	26	89	...	...	115	...
,,	,,	320	87	81—91	11	83	...	...	115	...
,,	,,	333	91	84—97	14	88	...	...	116	...
,,	,,	336	96	84—103	20	92	...	...	103	...
,,	,,	404	90	78—106	29	90	...	...	107	...
,,	,,	433	98	81—112	32	93	...	...	90	...
,,	,,	486	98	81—91	11	90	...	...	115	...
,,	,,	546	87	81—91	11	85	...	...	114	...
,,	,,	583	87	78—106	29	87	...	...	128	...
,,	,,	869	93	81—91	11	87	...	...	118	...
Late .	Segregating.	12	126	...	...	...	25	88	113	22·1
,,	,,	61	121	...	...	...	28	74	102	27·5
,,	,,	62	123	...	...	...	31	87	118	26·3
,,	,,	187	120	...	...	...	29	69	98	29·6

*The term pure is used here not in the sense of genetic purity (homozygosity), but only to distinguish F_3 families which gave only late or only early plants from those which threw both types of plants.

TABLE III—*contd.*

Nature of F_2 parents	Segregation in F_2	F_3 Family No.	F_3 Duration	F_3 Flowering			Segregating Families		Total No. of Plants	Percentage of early plants
				Range	Period	Mean Duration	Earlies	Lates		
Late	Segregating.	216	125	...	...	...	43	99	142	30·3
,,	,,	256	121	...	...	...	29	86	115	25·2
,,	,,	316	119	...	...	...	37	79	116	31·9
,,	,,	346	118	...	...	...	27	90	117	23·1
,,	,,	377	121	...	...	...	23	89	126	20·5
,,	,,	398	123	...	...	...	30	87	117	25·6
,,	,,	494	121	...	...	...	23	103	161	18·2
,,	,,	516	122	...	...	...	17	92	109	15·6
,,	,,	527	127	...	...	...	36	78	114	31·6
,,	,,	610	126	...	...	...	33	85	118	28·0
,,	,,	670	120	...	...	...	29	88	117	24·8
,,	,,	747	123	...	...	...	17	99	116	14·7
,,	,,	806	121	...	...	...	19	99	118	16·1
,,	,,	847	117	...	...	...	26	87	113	23·0
,,	Pure late	2	130	129—139	11	133	...	...	93	...
,,	,,	21	130	123—136	14	129	...	...	116	...
,,	,,	27	134	126—142	17	135	...	...	93	...
,,	,,	46	123	117—142	26	124	...	...	115	...
,,	,,	66	130	129—133	5	132	...	...	113	...
,,	,,	121	123	120—130	11	125	...	...	115	...
,,	,,	151	121	117—130	14	123	...	...	115	...
,,	,,	179	128	129—148	20	133	...	...	112	...
,,	,,	219	129	129—136	8	133	...	...	111	...
,,	,,	244	121	114—124	11	119	...	...	114	...
,,	,,	334	127	129—139	11	134	...	...	115	...
,,	,,	351	132	129—148	20	140	...	...	116	...
,,	,,	373	126	120—136	17	127	...	...	95	...
,,	,,	397	129	129—136	8	136	...	...	108	...
,,	,,	485	126	114—133	20	124	...	...	120	...
,,	,,	561	127	123—133	11	127	...	...	118	...
,,	,,	895	127	126—133	8	130	...	...	103	...

The segregation into early and late plants was, in every case, very definite and clear-cut, the interval between the upper extreme of early group and the lower extreme of late group being in most families, well over a week. Intervals of two or

three weeks were common. In fact in many of the families some of the early segregates were almost ripe before the late ones started flowering. The classification of plants into earlies and lates in the segregating families was thus very easy, the rather long period of lull leaving no element of doubt regarding the accuracy of the classification.

The interval between the beginning and the finish of flowering in a culture and referred to as flowering period in Table III (Column 6) gives a true picture of the homozygosity of a family. Those which had flowering periods greater than those of the pure lines may be called heterozygous. The pure lines have been observed to take usually from seven to ten days to complete the flowering, though sometimes due to climatic or soil factors the period may be extended to fourteen days or even more. A period of thirteen days or less should be taken as an index of homozygosity, as the observations with pure lines as well as homozygous-looking hybrid families would lead us to assume. On this basis the number of homozygous families was found to be only twenty-three out of a total of 172, *i.e.*, 13·4 per cent of the whole ; of the forty-six early families only five were homozygous, *i.e.*, 10·9 per cent only. It will be shown later how the percentage of homozygous families increased rapidly from one generation to the next.

Relation between F_2 and F_3 durations

F_2-F_3 correlations.—An attempt was made to find out how far the variations observed in the F_2 generations were reproduced in F_3 ; in other words, it was desired to know how far the variation observed in F_2 was genetic. Information on such a problem is best given by the study of correlation between the two generations. Therefore, coefficients of correlation were studied between the F_2 flowering durations and the corresponding F_3 mean durations for both early and late families. Fig. 2 illustrates the correlation which exists between the two generations. In Fig. 2 each dot represents a family, its position indicating the mean value of the family and the duration of its F_2 parent. The arrangement of the dots indicates the existence of positive correlations in the early as well as the late families. The two coefficients and their standard errors are $+ 0{\cdot}4860 \pm 0{\cdot}114$ and $+0{\cdot}9254 \pm 0{\cdot}018$ for the early and late groups, respectively. The number of paired observations was forty-six in the case of the early families and sixty-six in the case of the late families. Reading the significance of the observed correlations from Table V A of Fisher [1932], we find that for $n=40$ or 60, these values show that P is less than 0·01 ; therefore, the correlations are definitely significant. They thus indicate the fact that the variation observed in F_2 was, in a great measure genetic. The wideness of space between the early and the late groups in Fig. 2 shows the difference in time of flowering in early and late families and recalls a similar wide difference existing in the parents themselves.

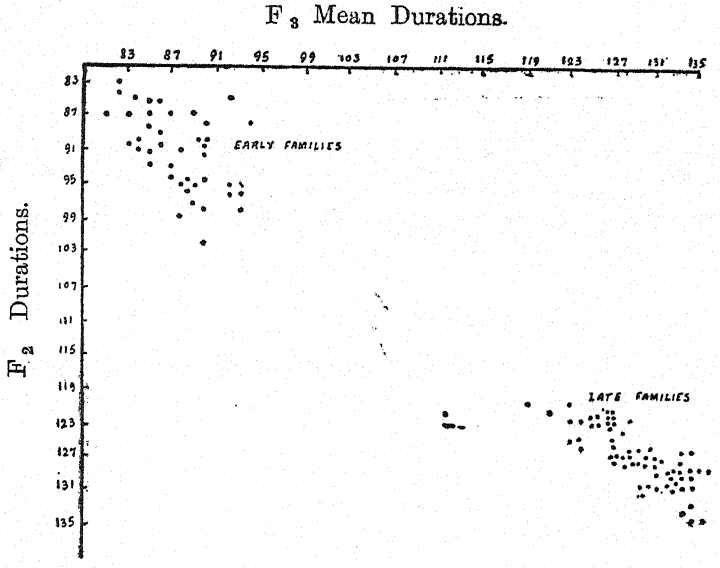

Fig. 2. Correlation between F_2 duration and F_3 mean durations in early and late families.

Fig. 2 is a very apposite illustration of parent-progeny correlation. Mention has been made of the inheritance of F_2 variation in F_3 in rice by other workers in India, but as to the intensity of the relation between the two generations, adequate data have not been presented so far. The magnitude of the work necessary for collecting such data is great and may account for the deficiency of such an information. The data given above seek to make up for this deficiency.

Relation between F_2 duration and F_3 behaviour

It has already been stated that the early F_2 segregates gave only early plants in F_3. Of the late segregates those that segregated into earlies and lates in F_3 flowered, on the whole, slightly earlier in F_2 than those which gave only late plants. In other words, the impure lates were, on the whole, earlier than the pure lates. This difference, though definite, is not clear-cut, because the ranges within which these two types of F_2 lates vary overlap each other considerably as will be seen from Table IV prepared from figures given in column 4 of Table III.

TABLE IV

Distribution of flowering duration of the selected F_2 late plants

Number of F_2 parents	Flowering duration in days																		Mean flowering duration	S. E. of Mean	Mean difference
	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134			
Impure lates.	2	3	2	3	8	15	10	10	1	1	4	3	1	1						121·72	0·369	5·17
Pure Lates						2	1	14	2	3	3	7	10	10	9	..	2	1	2	126·90	0·387	

The difference between the two groups, *viz.*, 5·17 days, is statistically significant, being about ten times the standard error, as shown below :—

$$\frac{\text{Difference}}{\text{S. E. of difference}} = \frac{5{\cdot}17}{\sqrt{0{\cdot}369^2 + 0{\cdot}387^2}} = 9{\cdot}667$$

This establishes the fact that the impure lates are, on the whole, earlier than the pure lates. The wide overlapping of the flowering duration figures in the two cases will be explained later.

How does the above relation between the constitution of the late F_2 hybrids and their durations affect the flowering data of the F_3 generation ? On a little thought it will become evident that such a relation will affect the flowering periods of the late hybrids of pure late and the segregating families in the following way. The late plants of the segregating families will be of two kinds, *viz.*, impure lates and pure lates. Now, since in a family the former tend to give shorter flowering durations than the latter, the result will be an extension of the flowering period of the late plants in the family. On the other hand, the flowering periods of the pure late families will be shorter as these families do not possess ' impure ' plants with a tendency towards shorter flowering duration. That this actually happened in F_3 was clear from a study of the various families as shown in Table V. It gives a comparison of the flowering periods of (1) the late plants from segregating families and (2) the late plants from ' pure ' late families.

TABLE V

Comparison of flowering periods of late plants from segregating and ' pure ' late families

Nature of F_3 families	Flowering period in days										No. of families	Mean flowering period	Mean difference
	5	8	11	14	17	20	23	26	29	32			
'Segregating'	..	1	..	6	10	21	13	7	2	..	60	20·35	5·12
' Pure '	2	4	14	16	15	10	2	2	..	1	66	15·23	

It will be seen that the mean flowering period of the 'segregating' families is greater (20·35) days than that of the 'pure' late families (15·23 days). The mean difference, *viz.*, 5·12 days is statistically significant (about 19·45 times its standard error) which is very high.

An explanation may now be given of the wide departure from expected numbers of the segregating and pure late families. It was stated on page 12 that the two kinds of families were 60 and 67 in number, respectively, although on a 3 : 1 basis the numbers ought to have been 85 and 42. The explanation is supplied by the fact given above, *viz.*, that the impure late plants have shorter flowering durations than pure late plants. The mean flowering duration of the late F_2 plants which were selected for the F_3 generation was greater than that of the unselected F_2 population of late plants. That means that the selected F_2 plants were in a greater number of cases 'pure' than would have been the case if the selection were quite random. The difference in the flowering durations of the selected and unselected F_2 late plants is shown in Table VI.

TABLE VI

Frequency distribution of unselected and selected late F_2 plants

F_2 plants	Flowering duration in days									Total	Mean duration
	115	118	121	124	127	130	133	136	139		
Unselected lates .	27	128	218	115	114	55	18	4	4	683	122·94
Selected lates . .	2	8	32	31	28	20	5	..	..	126	126·69

The mean difference in Table VI is quite significant. The mean duration of the selected late plants is almost the same as that of the 'pure' late plants given in Table IV, which is 126·90. The bias given to the selection whereby it included a greater number of pure late plants than would have occurred if chance alone operated explains why in the F_3 there was a preponderance of 'pure' late families.

The comparative precocity of the 'impure' late plants is of some practical significance. One of the aims of the cross was to evolve late types which were earlier than the late parent. Now, since the 'impure' late plants have a tendency to flower earlier, selection of the earlier plants from 'segregating' families in the F_3 or later generations would almost always result in segregation into early and late plants in the next generation. Hence, during the selections this fact had to be borne in mind, and greater importance was attached to the earlier plants of the 'pure' late families than to those from 'segregating' families.

F_4 generation.—Fifty-two early F_3 plants and sixty-one late F_3 plants were selected for raising the F_4 generation. The early plants again gave families consisting of early plants only. The flowering periods of the families were, however,

shorter than those of the F_3 families. The average for the fifty-two F_4 families was 15·50 days, while the average for the F_3 families from which the parents for these F_4 families came was 18·96 days—a difference of 3·46 days. An examination of the figures shows that this difference is due both to an increased number of pure (homozygous) families in F_4 as well as to the reduction of flowering periods of impure (heterozygous) families.

Of sixty-one late selections, thirty-six gave only late plants in F_4, while twenty-five segregated into late and early plants. The total frequencies of the latter are :—

	Lates	Earlies	Total
Observed	964	350	1,314
Expected on 3 :1 basis	985·5	328·5	

Deviation . 21·5

S. E. . . 15·7

$\frac{\text{Deviation}}{\text{S. E.}} = 1{\cdot}369$

i.e., the frequencies are according to expectation.

The number of homozygous families had increased considerably. For example, of the fifty-two early F_4 families, seventeen had flowering periods of thirteen days or less, *i.e.*, they were pure for flowering. That comes to 32·7 per cent of the total number of early families as against 10·9 per cent of early families in F_3 (page 1254), *i.e.*, an increase of about 300 per cent.

In the F_4 stage families with very narrow range of flowering as well as with wide range were obtained. Segregation had, besides, produced families with distinctly different mean flowering durations. A few of the families are illustrated in Fig. 3 which shows the flowering distribution of four early F_4 families, *viz.*, 70-66, 220-3, 14-45 and 512-91, the last of which shows a narrow range and is pure while the first one is distinctly impure. The other two are showing shorter ranges than family 70-66 but are impure.

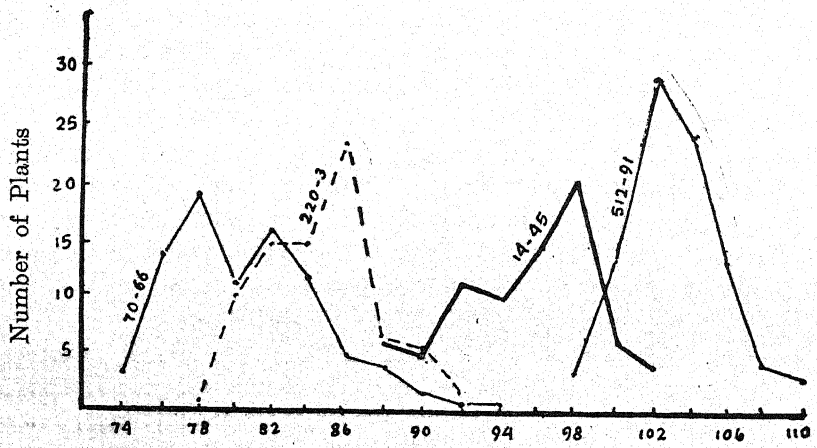

Fig. 3. Four F_4 early families with different mean durations.

F_5 generation.—In this generation there was a further increase in homozygosity. Out of thirteen F_5 families derived from early F_4 families, six were homozygous for flowering, showing ranges of seven to ten days while one with a range of thirteen days was of doubtful homozygosity. The rest possessed flowering periods of fifteen to seventeen days. The mean period for these thirteen F_5 families was 12·0 days, compared to 15·1 days, the mean flowering period of the corresponding F_4 families.

GENETICS OF THE CROSSES

The foregoing facts can be explained by the following scheme of inheritance. The early type is assumed to have the constitution **aaBBcc** and the late type **AAbbCC**. **C** is the factor for lateness which completely dominates over **A**, a factor for earliness present in the late type. **B** is another factor for earliness present in the early types. This is also recessive to **C** but not so completely as **A**, and gives with **C** plants slightly earlier than the late parent. The cross can be represented thus :

		T. 1		T. 110
		aaBBcc		**AAbbCC**
F_1	.....		**AaBbCc**	
F_2		27	**A B C**	Late
		9	**A B c**	Early
		9	**A b C**	Late
		9	**a B C**	Late
		3	**A b c**	Early
		3	**a B c**	Early
		3	**a b C**	Late
		1	**a b c**	Early

Thus lates : earlies are 48 : 16, or 3 : 1. F_1 is slightly earlier than T. 110, because of the presence of **B**. Similarly in F_2 the majority of lates possess the factor **B**, *viz.* 27 **A B C** and 9 **a B C**, and are consequently slightly earlier than T. 110. The 'shift' in the late segregates towards the middle can thus be accounted for. Similarly the shift in the early segregates can be explained if we assume that the factor **A** for earliness causes a slightly delayed earliness than that of T. 1, both alone and in the presence of **B**. Thus in the scheme out of the sixteen earlies, twelve possess the factor **A** and are, therefore, later than T. 1.

Before explaining the data on the basis of this theory let us first analyse the behaviour of the different genotypes.

The twenty-seven different genotypes, consisting of sixty-four F_2 individuals will breed in several different ways. Classifying them broadly in to three divisions,

viz. (*i*) those which give only late plants, (*ii*) those which give early as well as late plants (*iii*) those which give only early plants in F_3, they can be arranged as below :

(i) Genotypes which give only late plants in F_3	(ii) Genotypes which segregate in F_3 into early and late plants	(iii) Genotypes which give only early plants in F_3
1. (a) 1 **AABBCC**	1 (b) 2 **AABBCc**	2. (a) 1 **AABBcc**
1. (c) 2 **AABbCC**	1. (e) 4 **AABbCc**	2. (b) 2 **AABbcc**
1. (d) 2 **AaBBCC**	1. (f) 4 **AaBBCc**	2. (c) 2 **AaBBcc**
1. (g) 4 **AaBbCC**	1. (h) 8 **AaBbCc**	2. (d) 4 **AaBbcc**
3. (a) 1 **AAbbCC**	3. (b) 2 **AAbbCc**	5. (a) 1 **AAbbcc**
3. (c) 2 **AabbCC**	3. (d) 4 **AabbCc**	5. (b) 2 **Aabbcc**
4. (a) 1 **aaBBCC**	4. (b) 2 **aaBBCc**	6. (a) 1 **aaBBcc**
4. (c) 2 **aaBbCC**	4. (d) 4 **aaBbCc**	6. (b) 2 **aaBbcc**
7. (a) 1 **aabbCC**	7. (b) 2 **aabbCc**	8. 1 **aabbcc**
= 16 individuals	= 32 individuals	= 16 individuals

Thus about twenty-five per cent of the F_2 plants should give only late plants in F_3, and fifty per cent should segregate in F_3. This relation was not realized in the F_3 and the reason has already been given on page 1257, *viz.* that the selection of F_2 plants was not random, for it contained a greater number of 'pure' late plants, than would happen if chance alone operated. As a proof it was stated that the mean flowering duration of 'pure' late F_2 plants was greater than that of the 'impure' late plants, and that the selected plants had a flowering duration almost identical with that of the former. The shorter flowering duration of 'impure' late plants is due to the fact that they comprise genotypes which have only one dose of the factor **C**, besides most of them possessing the factor **B** [column (ii) of preceding statement]. The presence of the **B** factor in the genotypes consisting of pure late plants [column (i) in the preceding statement] also explains the wide overlapping in the flowering duration—figures of the two types of late plants, *viz.* the 'pure' and 'impure' lates,—a fact already stated (page 1256). For, as stated on page 1259, **B** is a factor which gives the late plants a shorter flowering duration because of its incomplete recessiveness to the factor **C**. Now, six of the genotypes of the 'pure' late group contain the

factor **B**, hence they have flowering durations nearly as short as that of the 'impure' lates. On this assumption we should expect, in F_3 or later generations, true breeding late families with flowering durations appreciably shorter than that of T. 110. Such families will apparently have the genetic constitution **AABBCC** or **aaBBCC**. In Table III (page 1253), families 121, 244, 561, etc. belong to this group since their mean durations are, respectively, 125, 119 and 127 days and their flowering periods short enough to mark the families as pure. Such families fulfil one of the important aims for which the cross was made, *viz.* evolution of late types with durations shorter than that of T. 110.

Although the term 'pure' has been generally used for individuals giving only early or late plants, it should not be confused with homozygosity. Many of these so called 'pure' plants were heterozygous. Taking the late families first, it is clear that plants with a constitution, say, **AABbCC**, will give only late plants, but they will be of two different kinds, one having the duration of F_1 (*i.e.*, earlier than T. 110) and the other having the duration of T. 110. This segregation will consequently increase the flowering period of the family appreciably. Similarly in the case of the early families, those containing plants of the constitution **AAbbcc** or **AABBcc** will be later than the parent although pure in the sense that they consist of homozygous plants. On the other hand families coming from plants of the constitution **AaBBcc** or **AaBbcc** will consist of plants of different kinds some containing the factor **A** and some without this factor, thus being of different durations. Such families are, in the real sense of the term, splitting.

The triple recessive hybrids :—Although in the above cross an early type (T. 1) was crossed with a late one (T. 110) some of the segregates in F_3, F_4 and F_5 were earlier than the early parent. In the scheme of inheritance advanced above this earliness of the hybrids can be explained only if we assume that they are the triple recessive hybrids. Since the selections in the F_2 and the F_3 generations were mainly guided by economic considerations and since some of the earliest plants were very poor in promise they were not included among the selections, and the fact of the extra earliness of the triple recessives could not be very well ascertained at first. The only evidence forthcoming was that certain plants in the F_3, F_4 and F_5 generations flowered earlier than the T. 1 plants. This could only happen if their earliness could be ascribed to the absence of all the three factors **A**, **B** and **C**. If this were really so, and if corresponding selections were made in F_2, we should have got at least one or two pure early families, showing a duration shorter than that of T. 1. Fortunately a duplicate cross had been made in 1931 from which an F_2 was grown in 1933. Selected plants from this were used for raising an F_3, the study of which has amply proved the above view. A part of the data is given in Table VII.

TABLE

Frequency distribution of flowering duration

Flowering

Generation	Year	Culture No.	75 76	77 78	79 80	81 82	83 84	85 86	87 88	89 90	91 92	93 94	95 96	97 98	99 100	101 102	103 104	105 106
P_0 .	1933	T. 1 . .						15	40	25	7	7						
P_0 .	,,	T. 110 .																
F_2 .	,,	F_2 . .				1	2	1	9	26	37	43	39	15	7	3	5	1
P_0 .	1934	T. 1 . .					5	14	52	30	5	1						
P_0 .	,,	T. 110 .																
F_2 .	,,	39				2	5	24	34	27	21	4						
,, .	,,	46			3	13	8	29	41	17	10	1						
,, .	,,	53	2	19	44	33	8	5	5									
,, .	,,	57				2	2	10	13	36	28	19	3	2		1		
,, .	,,	77		3	36	34	12	24	4	3								
,, .	,,	139				2	2	8	23	38	31	7	4					
,, .	,,	196										1		2	4	9	21	23
,, .	,,	245					1		10	14	18	22	4	14	2	3	6	4
,, .	,,	448	7	31	58	14	7	2										
,, .	,,	496			1	6	6	21	49	18	12	1						
,, .	,,	141					6	6	10.	1								
,, .	,,	201				1	1		11		4		1	1				
,, .	,,	253		1	1	2		1	9		9	1					1	
,, .	,,	512				1		1	7	2	6	5	4	3	1	3	3	
,, .	,,	590						2	5	2	3	6	4	2		1	3	
,, .	,,	611				1	5	6	9	4	4	2	3					
,, .	,,	466																
,, .	,,	519																

VII

in parental, F_2 and F_3 generations

Duration

107 108	109 110	111 112	113 114	115 116	117 118	119 120	121 122	123 124	125 126	127 128	129 130	131 132	133 134	135 136	137 138	139 140	141 142	143 144	145 146	147 148	149 150	151 152
														6	29	34	10					
		1	1	8	19	73	84	64	46	18	99	30	26	19	13	5	3	1				
																1	3	13	93			
15	18	5																				
4	3																					
		1	2	37	12	13	1	8	2	7	3	1										
				1	3	11	3	11	7	9	8	1	5	5	2	5	7	4	6			
					3	7		8	15	12	6	3	3	4	7	3	3	6	4	44	1	
							2		1		2	6	12	21	13	16	5	1	3		1	
									7	9	18	17	12	9	6	7	3		3			
								4	7	9	16	6	11	8	5	8			2			
										9	13	14	17	23	8	12	5	5	4			
																4	10	19	35	4	10	4

In Table VII, some of the F_3 families bred from F_2 plants of different genotypic constitutions are shown. Apparently F_3 families, No. 53 and No. 448, are from the triple recessive hybrids of constitution **aabbcc** because these families are earlier than Type 1. Family 466 is from an F_2 plant of constitution **AABBCC** and consists only of late plants, most of them earlier than Type 110. Family 519 is from a plant of constitution **AAbbCC** or **AabbCC** and gives a duration more or less like that of T. 110. Of the segregating families, family 611 must be from a plant of constitution **AaBBCc** because the early segregates have durations of T. 1 as well as longer durations and the late segregates are mostly earlier than T. 110. This fits in with the conditions demanded by the theory advanced. Similarly family 253 must have come from a plant of constitution **AabbCc**.

The explanation of the genetics of the cross fits in satisfactorily with the facts observed. It should, however, be noted that the phenotypic classification of the hybrids in F_2 and later generations is not as simple as would be expected on a tri-hybrid basis, because the flowering duration is a character which is not characterised by a clear-cut segregation usual with qualitative characters. The fluctuation shown by the character causes the different phenotypes to merge into each other, thus making it difficult to classify them clearly. Yet the genetical behaviour of the cross is such as to admit of the action of only three important factors in spite of the fluctuations present. That the three factors do not represent the complete picture of the cross is evidenced by the study of later generations. The minute differences between families and the very gradual narrowing of flowering periods in successive generations, are proofs that besides the three major factors already described there are several minor factors deciding the heredity in the cross. The tri-hybrid explanation advanced has, therefore, to be taken with this reservation.

Other economic crosses.—In essential features the data from other crosses were similar to those for the cross already described. In every case the F_2 and F_3 generations were studied in detail, record for individual plants being kept separately. In F_4 and F_5 generations, the purity or otherwise of the different families was noted by repeated countings of the flowered plants on alternate days. On account of the similarity of the results with those already described in detail for cross No. 23 (T. 1 × T. 110) it will be unnecessary to give the details here. Only the broad outlines of the results are presented.

F_2 generations.—They showed clear segregation into early and late plants. Unlike cross 23 described above the other crosses gave good fits of 3 late : 1 early plants. The population varied from 600 to 900 plants. The crosses studied were :

Cross No.	Parents
21	T. 1 and T. 100
24	T. 3 and T. 5
25	T. 1 and T. 26
27	T. 12 and T. 26

Types 5, 26 and 100 were the late parents.

F_3 generations.—The early F_2 plants bred only early plants in F_3. The late F_2 plants either bred 'pure' or segregated into earlies and lates. The number of F_2 plants selected and their behaviour in F_3 are given in Table VIII.

TABLE VIII

F_2 plants selected and their behaviour in F_3

Cross No.	No. of F_2 families	Nature of F_2 parents	Segregation	Frequencies	
				Calculated	Observed
22	221	55 Early . 166 late . .	'pure' early 'pure' late segregating into 3 lates : 1 early	55 55 111	55 64 102
25	209	36 early . 173 late .	'pure' early 'pure' late segregating into 3 lates : 1 early	36 58 115	36 73 100
27	153	39 early . 114 late .	'pure' early 'pure' late segregating into 3 lates : 1 early	39 38 76	39 68 46

The frequencies of the early and late classes are given in Table IX.

TABLE IX

Early and late plants from segregating F_3 families

Cross No.	No. of families	Observed or calculated	Frequencies		Deviation	Standard Error	Dev./S. E.
			Early	Late			
22	102	Observed Calculated	2985 2903·75	8630 8711·25	81·25	46·66	1·74
25	100	Observed Calculated	2746 2698·5	8048 8095·5	47·5	44·98	1·06
27	46	Observed Calculated	1210 1221	3674 3663	11	30·26	0·36

It will be seen that the deviation in all the cases is within range of expectation.

The percentage of the early plants on the total in each family was separately worked out in the different crosses. It was seen that the percentages varied considerably and were, in a majority of cases, between twenty and thirty. Thus in

crosses 22, 23, 25 and 27, the number of families which had percentages of early plants between twenty and thirty was 205 out of 308. The mean percentages in the four crosses were 25·8, 24·2, 25·4 and 24·9, respectively—figures very near 25·0, the required theoretical figure.

F_4 generations.—The early F_3 plants again bred true to early habit in F_4. There were 402 early families in the crosses. The late F_3 plants which segregated in F_4 always threw early and late plants in 1 : 3 ratio. The frequencies and deviations are given in Table X. The number of 'pure' late families was 249 excluding the 36 families of cross No. 23, already mentioned.

TABLE X

Frequencies and deviations from calculated figures of early and late plants in segregating F_4 families

Cross No.	No. of families	Observed or calculated	Frequencies. Early	Frequencies. Late	Deviation	Standard Error	Dev. / S. E.
21	63	Observed	1047	2958			
		Calculated	1001·25	3003·75	45·75	27·40	1·67
22	41	Observed	648	1918			
		Calculated	641·5	1924·5	6·5	21·93	0·30
25	34	Observed	660	1845			
		Calculated	626·5	1878·75	33·75	21·66	1·56
27	22	Observed	458	1470			
		Calculated	482	1446	24	19·01	1·26

It will be seen that the ratio of early and late plants shows good fit in every case. The 'pure' families had become homozygous to a considerable extent as already described in cross No. 23.

F_5 generations.—There was a total of 132 early families in all the crosses, 35 families segregated into early and late plants, and 65 families gave only late plants. The frequencies of early and late plants from the 35 segregating families are as follows :

	Early plants	Late plants
Observed	951	2915
Calculated	966·5	2899·5
Deviation	15·5	

$$\text{Deviation/S. E.} = \frac{15\cdot5}{26\cdot7} = 0\cdot6 \text{ (nearly)}$$

CORRELATION BETWEEN FLOWERING AND SOME OTHER CHARACTERS

A few interesting correlations between flowering duration and other characters may now be described.

1. Flowering and yield.—Yield is a very important consideration in crop improvement. Since, however, it can not be judged with any degree of completeness in the earlier stages of breeding work the utmost that a worker can do is to exploit its correlations with the more easily recognisable features of the crop and thereby be guided in his selections. Securing a marked earliness alone would not be an achievement unless it were accompanied by good yield. The importance of the two characters, *viz.*, earliness and yield is obvious. Until the two characters are studied in relation to each other selection is likely to be arbitrary. In the collection of pure lines at the Rice Research Station, Nagina, it has been observed that some of the late types appear to be of remarkable yielding capacities. There are no doubt a few early heavy yielders, but the balance of heaviness seems to be inclined towards the late types. No correlation between flowering duration and yield has been studied in the pure lines. In the crosses, however, it was suspected that a positive correlation might be present in which case it would become difficult to combine earliness with very high yields in the hybrid varieties. It was, therefore, felt that a knowledge of the correlations would enable us to judge the possibility of combining the two characteristics, and to base our selections on surer foundations. The results are given below :—

In 1933 an F_2 generation of cross No. 23 (T. 1 × T. 110) was harvested, grain from individual plants was threshed separately, and after the grain was thoroughly dried in the sun the weights were taken in grammes. The correlation between yield of individual plants and the flowering duration was calculated separately in the early and the late groups. The values of r and their standard errors were $+0{\cdot}0268 \pm 0{\cdot}0808$ and $+0{\cdot}0348 \pm 0{\cdot}0472$, respectively for the two groups. Apparently correlation was absent. The mean yield of the late hybrids was, however, slightly greater than that of the early hybrids, but the difference was not statistically significant. The yield distribution of the hybrids transgressed the parental limits in both the directions, especially toward the higher extreme. The four mean values are given below :

Culture	Mean yield (in grms.)
T. 1	25·89
T. 110	33·89
F_2 Early	45·43
F_2 Late	47·14

The markedly higher figures in the hybrids may be due to hybrid vigour. The mean difference between the parents, *viz.*, 8·0 grms. is statistically significant.

2. Average yield per ear.—One of the factors which determines the yield of crop is the heaviness of individual ears of a plant. An average ear of T. 110 possesses

a very much greater number of spikelets than an ear of T. 1, resulting in a greater weight of threshed grain from each ear. It was resolved to investigate if the relation shown by the parents, *viz.* greater yield per average ear in late plants (T. 110) compared to that of early plants (T. 1), was reproduced in the F_2 hybrids too.

In F_2 the coefficients of correlation between flowering duration and yield per ear were calculated separately for the early and the late hybrids. The two values of r are $+0{\cdot}0457$ and $-0{\cdot}0152$. None of these values is significant. That is, within the same group, the later plants do not give heavier ears. However, the mean yield per ear of the late group is greater than that of the early group, the two means being 3·22 and 2·95 grms., respectively. The difference between the two figures has been found to be statistically significant, the value of 't' being 4·05.

The comparison of hybrids with the parents for the character under consideration is given in Table XI.

TABLE XI

Frequency distribution of one-ear yield in parents and F_2 hybrids of cross T. 1 × T. 110 in 1933

Class centres in grms.	T. 1 (1)	T. 1 (2)	T. 110 (1)	T. 110 (2)	F_2 hybrids	
					Early	Late
0·9	..	..	..	..	1	3
1·1	..	1	..	..	..	3
1·3	..	..	..	..	1	2
1·5	..	..	..	..	1	5
1·7	3	3	..	..	3	11
1·9	2	1	..	1	4	11
2·1	5	16	..	1	9	17
2·3	7	9	2	1	11	23
2·5	13	18	2	2	25	32
2·7	13	14	3	..	15	38
2·9	14	14	7	8	14	41
3·1	9	3	11	5	20	43
3·3	8	1	11	14	16	44
3·5	1	1	8	15	9	45
3·7	..	..	10	11	9	35
3·9	..	..	17	16	11	33
4·1	..	..	13	13	7	19
4·3	..	..	7	7	1	17
4·5	..	..	8	8	..	13
4·7	..	..	7	1	2	9
4·9	..	..	5	..	..	12
5·1	..	..	..	1	..	2
5·3	..	..	..	..	..	3
5·5	..	..	..	..	..	1
5·7	..	..	..	..	..	..
5·9	..	..	..	..	..	1
6·1	..	..	..	..	..	1

It will be seen that the distribution in T. 110 occupies higher classes than that in T. 1. In each type distributions of two cultures are given, one grown on each side of the hybrid lines in the field. The means of the four parent cultures and the two hybrid groups are as follows :

T. 1 (1)	2·6867 grms.
T. 1 (2)	2·4926 „
T. 110 (1)	3·7523 „
T. 110 (2)	3·6635 „
F_2 Early	2·9503 „
F_2 Late	3·2203 „

The mean difference between the early parent and the F_2 early group, and that between the late parent and F_2 late group are statistically significant, the F_2 early and late groups being intermediate between the means of the two parents. This is only a case of ' shift ' in inheritance of the character.

The frequency distributions of the hybrids, besides showing mean values intermediate between the parents, are showing greater ranges of variation than the range occupied by the parents. Thus the hybrid plants show both lighter and heavier ears than the parental extremes. The former may be due to an abundance of sterile spikelets possessed by some of the hybrids and the latter may be due to hybrid vigour.

3. Weight of five ears.—In the above study the average yield per ear was obtained by dividing the plant yield by the number of ears harvested. The inheritance of heaviness was studied by a different method in another F_2 culture of the same cross in 1931. In this case, weight of five ears was taken as a character for study. The parental and F_2 cultures were studied in the same year.

The mean weight, range of variation, and standard error of mean weight are presented in Table XII.

TABLE XII

Mean five-ear weight, range of variation, etc. in parental and F_2 cultures in 1931

Culture	No. of plants	Range of variation grms.	Mean weight grms.	S. E. of mean
T. 1	130	15·5-29·5	23·50	0·1894
T. 110	130	23·5-41·5	34·44	0·2836
F_2 Early	182	5·5-33·5	20·03	0·4338
F_2 Late	683	5·5-51·5	22·54	0·3070

The two F_2 groups, early and late, show variation over a wide range, and show a difference in mean weights which is statistically significant. The coefficients of correlation between the flowering duration and five-ear weight were calculated

separately for early and late hybrids, and are, respectively, —0·1741 and +0·4529. Fisher's Table VA [Fisher, 1932] shows that the former value is not significant, the value of P being above 0·05; the latter value is significant, the value of P being less than 0·01.

The distribution of five-ear weights, like the distribution of one-ear yield, was very wide in F_2 and embraced both the extremes of the parents, and even extended beyond. The early plants possessed, on the whole, lighter ears than the late plants. In F_2, F_3 and F_4, certain plants were obtained in the early as well as the late groups which possessed heavier ears than the heaviest-eared plants of the parents. The distributions of parents, F_2 and the selected plants of F_2, F_3 and F_4 are illustrated in Fig. 4 where the barred columns represent early plants and the solid columns, the late plants.

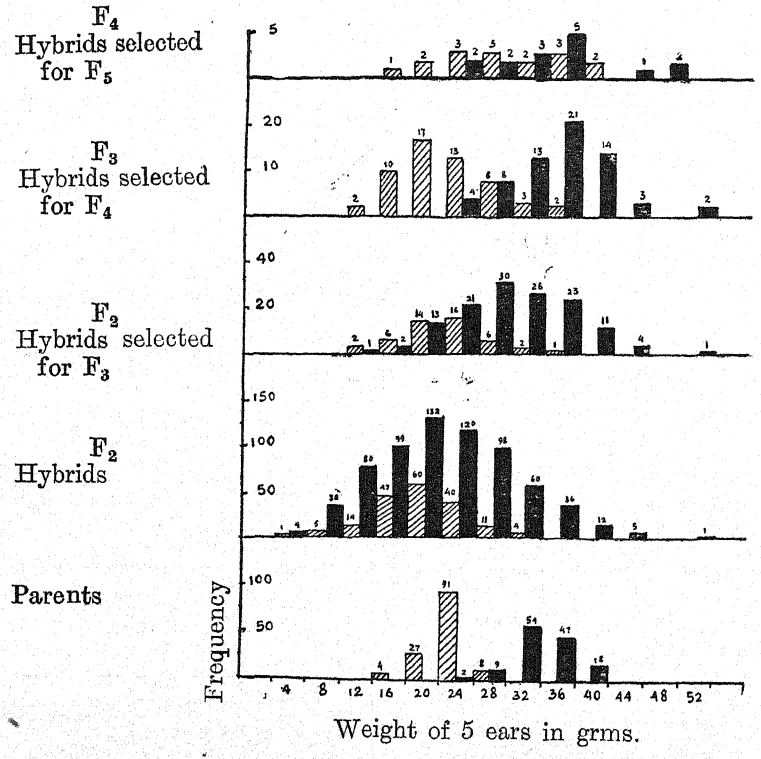

Fig. 4. Weight of five ears in parental, F_2, F_3 and F_4 generations.

4. Flowering and number of tillers.—Tillers were counted in the parents and the F_2 generation in a cross between T. 1 and T. 110 in 1933. Each parent was represented by two cultures in the field, one on each side of the hybrid lines. The range

of variation was greater in the F_2 than in the parents. The averages and their standard errors are given below.

Culture	Mean number of tillers	Standard error
T. 1 (1)	9·95	0·2373
T. 1 (2)	10·66	0·2786
T. 110 (1)	9·13	0·2489
T. 110 (2)	11·68	0·3251
F_2 Early	15·07	0·3418
F_2 Late	14·70	0·2148

The averages for the hybrids are appreciably higher than those for the parents. This may be only an expression of hybrid vigour.

The coefficients of correlation between the flowering durations and the number of tillers were also calculated both in the early as well as the late hybrids. The values of r obtained were, respectively, —0·0546 and —0·0258, thus showing a complete lack of correlation between the two characters. In other words the early plants are as likely to be well tillered as the late plants. Since tillering decides, to a considerable extent, the yield of a plant, the natural corollary of the above fact is that if once good tillering is secured in early cultures (which fact is possible because of the absence of correlation between flowering and tillering) there are reasonable chances of their being good yielders.

Summary

Some crosses between early and late types of rice have been studied for the inheritance of flowering duration and its relation to certain other characters, and the data have been presented in the present paper. The inheritance of flowering duration was thoroughly Mendelian. The main facts were :

1. The F_1 showed dominance of lateness, being only slightly earlier than the late parent.
2. The F_2 distribution covered the parental extremes, and could be divided into two distinct groups of hybrids, an early and a late group in the ratio of 1 : 3 thus indicating a dominance of lateness.
3. The two F_2 means were statistically different from the parental means, the former showing a ' shift ' toward the middle.
4. The segregations in F_3 confirmed the observations made in F_2. The segregation into early and late plants was always in 1 : 3 ratio, wide departures from the ratio being very few.

5. The correlation between the flowering durations of F_2 and F_3 generations was close and positive.

6. There was a definite relation between the flowering durations of late F_2 plants and their genetic constitution (as revealed by their behaviour in F_3), the impure lates being, on the whole, slightly earlier than the pure lates, the difference being statistically significant, although the flowering distributions of the two kinds of late plants showed considerable overlapping.

7. As a result of the relation mentioned in paragraph 6 of the range of flowering of late plants of 'segregating' families becomes greater than that of the late plants of 'pure' families in F_3 and later generations.

8. The number of homozygous families increased rapidly from generation to generation.

9. The genetics of the crosses have been explained on the assumption that three factors, one from the early types and two from the late types, interact in inheritance and cause the segregations observed. The facts mentioned in the preceding paragraphs have been satisfactorily explained by the tri-hybrid scheme of inheritance suggested. The scheme has, however, to be accepted with the reservation that besides these three important factors, there may be minor factors too which decide the inheritance in the cross.

10. Although the yield per plant was higher in the late parent, the F_2 late hybrids were not heavier in yield than the early F_2 hybrids. The coefficients of correlation between flowering and yield in the two groups were insignificant.

11. Yield per ear was greater in the late hybrids than in the early ones, as was the case in the parents.

12. The coefficient of correlation between flowering duration and weight of five ears was insignificant in the early F_2 hybrids, but significant in the late hybrids.

13. The parents differed from each other but slightly in the number of tillers per plant. The F_2 early and late groups possessed significantly greater number of tillers than the parents, but the difference between the two groups was not significant. The coefficients of correlation between flowering duration and tillering were insignificant in the two cases.

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MENDELIAN SEGREGATION IN *HIBISCUS SABDARIFFA* L. IN RELATION TO THE AGE OF THE F_1 AT TIME OF FERTILIZATION

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(With seven text-figures)

CONTENTS.

		Pages
I.	Introduction	1274
II.	Material and method	1275
III.	First series of experiments	1276
IV.	Second series of experiments	1287
V.	Discussion	1290
VI.	Summary and conclusion	1291
VII.	References	1291

I. Introduction

In the course of inheritance studies it is sometimes found that Mendelian ratios in the F_2 generation show a considerable deviation from theory. This is most frequently due to the population examined being insufficiently large. Other possible causes are linkage, lethal factors, or faulty identification of the phenotypes.

Various workers have brought forward evidence of the existence of yet another factor—a seasonal effect on segregation. Christie and Wriedt [1930], working with the domestic pigeon, found a seasonal variation in the segregation of the sex-linked factor for colour intensity. The same workers report that when hybrid Plymouth Rock × Minorka males (heterozygous for the sex-linked factor producing barring) were crossed with black Minorka females, there was a tendency to give an excess of barred offspring at the beginning of the season and an excess of black towards the end of the season. These instances suggest a seasonal variation in the viability or the power of fertilisation of the gametes. In the same material, however, Christie and Wriedt did not find any seasonal effect with regard to the sex-linked factor for piebald pattern, nor was any such effect noted by these workers on the segregation of the character pairs, greenness and yellowness, or tallness and dwarfness in *Pisum sativum*.

Haigh and Lochrie [1929], working with cowpea (*Vigna sinensis*), found a periodic variation in the Mendelian ratio between the dominant axil-spot plants and the recessive albino plants (3 : 1 on theory) in the F_2 progeny contributed by a number of F_1 plants selfed from day to day. They found that in the first-formed flowers there was a strong tendency to produce the recessives in excess, whilst in the progeny from later-produced flowers dominants became increasingly abundant until the ratio of dominants to recessives was the reverse of what was observed initially. This effect appeared to be unconnected with environmental factors such as temperature, humidity, etc., and is attributed by Haigh and Lochrie [1929] to the age of the parent.

If age has any influence on the F_2 segregation, then the stage at which the F_1 plants should be selfed, as also the extent of period for which they should be selfed, are very important points to consider in an inheritance study. It is these considerations which suggested the investigation described in the following pages.

II. Material and method

The plant selected for this investigation was *Hibiscus* (*H. sabdariffa* L.). The following considerations led to the selection of this plant :—

1. It provides sharply contrasting characters of which the mode of inheritance has already been studied.
2. The characters are such as can be studied when the plant is a month old or less. After the observations are over the crop can be ploughed in as a green manure and a winter crop raised in the same field the same year. This consideration is a great advantage in that it makes available the large area which is necessary for such an experiment.
3. Flowers are big and are easily selfed individually.
4. Seed production is high, each fruit normally giving about fifteen to twenty seeds. A maximum of about thirty-five seeds has been harvested from a single capsule.
5. The flowering period is of a fairly long duration.
6. The plant is practically free from insect pests and diseases.

Three Pusa types of *H. sabdariffa* L. were selected as parents of the crosses. These types have been maintained as pure cultures and their genetical constitution already studied [Howard and Howard, 1924]. Two characters, narrowness or broadness of leaf-lobe, and redness or greenness of stem-colour were taken into consideration for the present investigation. Narrow leaf-lobe is found to be dominant to its allelomorph, broad lobe, on a 3 : 1 monohybrid ratio. Red colour in stem in the cross, *New Hibiscus* $\times$ *Albus*, is dominant to green, giving a ratio of 3 reds (various grades and forms) : 1 green in F_2. This character in this cross is,

however, determined by three factors as shown by genetical investigations (unpublished records by the author). In the cross, *New Hibiscus* × *Ruber*, the red stem is dominant to green stem with red patches in the leaf-axil, etc., on a dihybrid basis, but the ratio of the frequency of the former phenotype to the latter is 3 : 1. In this investigation the counts were made on the above basis.

III. First series of experiments

The following crosses were made in the year 1931-32 :—

1. *New Hibiscus* × *Albus*
2. *New Hibiscus* × *Ruber*

New Hibiscus has narrow-lobed leaves and a green stem with red patches in the leaf-axil and just below it. *Albus* and *Ruber* have broad-lobed leaves ; but the former has a green stem and the latter red. The F_1, in both the crosses, had narrow-lobed leaves and red stem.

The seeds of the two crosses were sown the following year (1932-33), and a number of F_1 plants were raised. The spacing and subsequent treatments were kept uniform. Only five F_1 plants from each of the two crosses were kept, the rest being removed. All the ten plants were from seeds which germinated on the same day, and were uniformly vigorous and healthy from the beginning to the end.

The flowering commenced on November 2, 1932, in seven out of the ten F_1 plants and by November 5, all the plants were in flower. Thus, all these plants were also practically uniform as regards their flowering age.

(1) All flowers on each F_1 plant were selfed daily from the commencement of flowering till November 12, 1932, but thereafter with the increasing number of flowers, it was found difficult to self all of them, and hence only a sufficient number was selfed at random, the rest being pulled out.

(2) Selfing was done the evening previous to the opening of the flower by slipping a small wire ring over the conical bud. Rubber solution and " Seccotine " were also tried and found satisfactory.

(3) Each selfed bud was labelled, the label denoting the cross, the plant number, and the date of selfing.

(4) Every morning, at about 8 o'clock, which is the time for the opening of the flowers, the flowers were examined to see if the corolla had slipped out of the ring, in which case the flowers were rejected.

(5) Shedding was observed to take place. The number of flowers shed each day was recorded for each plant.

(6) As the fruits matured they were harvested. The produce of each plant, as also that from each day's selfing, was kept separate.

(7) Selfing was continued till the 25th December, 1932, and thereafter was stopped because of scanty flowering and low setting.

A complete record was kept of the following points :—

(*a*) the number of flowers selfed per day,
(*b*) the number of flowers removed per day,
(*c*) the number of flowers shed per day,
(*d*) the number of fruits harvested per day,
(*e*) the total number of flowers produced by each plant per day, and
(*f*) the date of first and last flowering.

Thus seeds from these F_1 plants selfed on successive days for about fifty to fifty-five days were available.

In the first week of July, 1933, these seeds were sown (dibbled) separately for each F_1 plant and for each day. Unfortunately the germination suffered due to heavy rains immediately after the sowing with the result that out of about 20,000 seeds sown only about 8,000 germinated.

Observations were taken when the plants were three to four weeks old. Even at this early stage the two characters, leaf-lobe and stem-colour, could be correctly classified into the different F_2 phenotypes.

In each of the two crosses, the following constants were calculated for both the characters (leaf-lobe and stem-colour) :—

(1) Ratio of dominants to recessives for each day.
(2) Deviation between the observed and theoretical frequencies of the recessive phenotype.
(3) Three times the Probable Error.

The ratio of dominants to recessives was first calculated for each F_1 plant separately, but as the different plants did not show any great variation in the behaviour for their segregation in F_2, the frequencies of all the F_1 plants of each cross were added up together.

Curves were drawn for each of the three above constants. Temperature and humidity curves for the period of flowering, as well as curves showing the total number of flowers produced per day for each cross, were also constructed. These showed no relation with the daily ratios and are not presented here.

Experimental results

The results of each cross will be dealt with separately.

New Hibiscus × *Albus*

Leaf-lobe.—New Hibiscus has a narrow leaf-lobe and *Albus* has a broad leaf-lobe. The F_1 had a narrow leaf-lobe. In F_2, a ratio of 3 narrow leaf-lobed plants : 1 broad leaf-lobed plant is theoretically expected in the population of each successive day, if age or any environmental factor were not to influence the F_2 segregation. The observed results are shown in the following table :—

TABLE I

Showing the daily frequencies of the two phenotypes, their ratios, total population, deviation between observed and theoretical frequency of the recessive, and 3 P. E.

New Hibiscus × Albus—F_2

Leaf-lobe

Flowering day	Narrow lobe	Broad lobe	Total	Ratio	Deviation	3 P. E.
1	27	7	34	3·86	—1·50	5·10
2	16	9	25	1·78	+2·75	4·38
3	52	15	67	3·46	—1·75	7·17
4	45	15	60	3·00	0·00	6·78
5	7	4	11	1·75	+1·25	2·91
6	94	30	124	3·13	—1·00	9·75
7	51	23	74	2·22	+4·50	7·53
8	75	24	99	3·13	—0·75	8·72
9	70	21	91	3·30	—1·75	8·35
10	224	85	309	2·63	+7·75	15·40
11	58	21	79	2·76	+1·25	7·79
12	87	31	118	2·81	+1·50	9·51
13	149	52	201	2·86	+1·75	12·39
14	133	35	168	3·80	—7·00	11·35
15	93	42	135	2·21	+8·25	10·18
16	101	40	141	2·52	+4·75	10·40
17	69	21	90	3·29	—1·50	8·31
18	120	39	159	3·08	—0·75	11·05
19	199	62	261	3·21	—3·25	14·16
20	119	42	161	2·83	+1·75	11·12
21	163	72	235	2·26	+13·25	13·43
22	116	43	159	2·70	+3·25	11·05
23	93	32	125	2·90	+0·75	9·79
24	80	23	103	3·48	—2·75	8·89
25	114	30	144	3·80	—6·00	10·51
26	84	45	129	1·87	+12·75	9·95
27	93	28	121	3·32	—2·25	9·64
28	69	23	92	3·00	0·00	8·41
29	39	11	50	3·82	—1·50	6·19
30	83	39	122	2·13	+8·50	9·68
31	59	25	84	2·46	+4·00	8·03
32	53	22	75	2·41	+3·25	7·59
33	69	30	99	2·30	+5·25	8·72
34	42	12	54	3·50	—1·50	6·44
35	89	42	131	2·12	+9·25	12·03
36	68	17	85	4·00	—4·25	8·08
37	4	2	6	2·00	+0·50	2·15
38	13	2	15	6·50	—1·75	3·39
39	8	2	10	4·00	—0·50	2·77
40				No setting		
41	6	2	8	3·00	0·00	2·48
42	19	7	26	2·71	+0·50	4·46

TABLE I—*contd.*

Flowering day	Narrow lobe	Broad lobe	Total	Ratio	Deviation	3 P. E.
43	1	1	2	1.00	+0·50	1·24
44	1	0	1	0·00	—0·25	0·88
45			No setting			
46	2	0	2	0·00	—0·50	1·24
47	4	2	6	2·00	+0·50	2·15
48			No setting			
49	5	2	7	2·50	+0·25	3·32
50	8	2	10	4·00	—0·50	2·77
51	9	4	13	2·25	+0·75	3·16
52	21	8	29	2·62	+0·75	4·72
53	43	11	54	3·91	—2·50	6·44
54	43	17	60	2·53	+2·00	6·79

$\chi^2 = 45 \cdot 25.$

$\sqrt{2\chi^2} - \sqrt{2n-1} = 0 \cdot 53.$ The fit is good.

Table 1 clearly shows that :—

(1) the ratio of the dominants to the recessives varies from day to day, but the positive and negative deviations appear fairly at random.

(2) the deviations on all days except one (26th day), *i.e.*, in about ninety-eight per cent cases are within the limits of chance errors and are thus not significant.

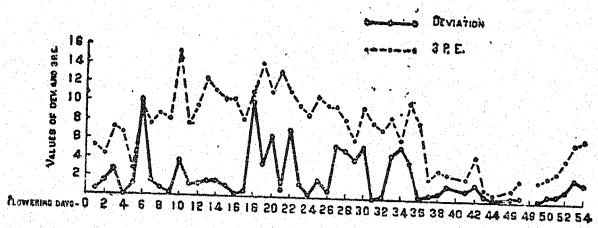

Fig. 1. Leaf-lobe (*New Hibiscus* × *Albus*).

The deviation curve (Fig. 1) will be seen to remain within the 3 P. E. curve all along except at one point, *viz.*, on the 26th, where it cuts the Probable Error curve, indicating a significant deviation in the ratio on that day only.

Thus it may be safely concluded from these results that the age of the F_1 plant or season has no effect on the F_2 segregation for this character (leaf-lobe) in *Hibiscus*.

Each individual F_1 plant showed a good fit to a 3 : 1 ratio when the daily frequencies were added up.

The total phenotypic frequencies in the whole cross were as follows :—

	Narrow-lobed plants	Broad-lobed plants	Total
Total observed	3,290	1,174	4,464
Total expected on 3 : 1 basis	3,348	1,116	4,464
Deviation	58	58	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{58}{19{\cdot}34} = 3.$$ The fit is just good.

The " fits " for the populations of all the five F_1 plants taken individually were good.

The " fit " for the frequencies during the whole flowering period was tested by the formula $\sqrt{2\chi^2} - \sqrt{2n-1}$ [Fisher, 1932] and was good as shown at the bottom of Table I.

Stem-colour.—New Hibiscus has a green stem with red patches in the leaf-axil and below it. The stem of *Albus* is purely green. The F_1 had red stem, and in F_2 the ratio of red stem (various grades) to green was as 3 : 1.

Table II and Fig. 2 show that in this case also there is no seasonal or age effect on segregation. The deviations between the observed and expected frequencies were, however, significant only on the 6th day.

TABLE II

Showing the daily frequencies of the two phenotypes, their ratios, total population, deviation between observed and theoretical frequency of the recessive, and 3 P. E.

New Hibiscus × *Albus*—F_2

Stem-colour

Flowering day	Red stem	Green stem	Total	Ratio	Deviation	3 P. E.
1	26	8	34	3·25	—0·50	5·10
2	18	8	26	2·25	+1·50	4·47
3	53	14	67	3·79	—2·75	7·18
4	45	15	60	3·00	0·00	6·78
5	7	4	11	1·75	+1·25	2·91
6	103	21	124	4·91	10·00	9·75
7	57	17	74	3·35	—1·50	7·53
8	75	24	99	3·12	—0·75	8·72
9	67	22	89	3·04	—0·25	8·26
10	228	81	309	2·81	+3·75	15·41
11	58	21	79	2·76	+1·25	7·79
12	88	31	119	2·84	+1·25	9·55

TABLE II—*contd.*

Flowering day	Red stem	Green stem	Total	Ratio	Deviation	3 P. E.
13	153	49	202	3·12	—1·50	12·44
14	129	41	170	3·15	—1·50	11·39
15	101	35	136	2·89	+1·00	10·25
16	106	35	141	3·03	—0·25	10·40
17	68	22	90	3·09	—0·50	8·31
18	110	50	160	2·20	+10·00	11·08
19	193	69	262	2·80	+3·50	14·19
20	115	47	162	3·19	+6·50	11·13
21	177	58	235	3·05	—0·75	13·43
22	112	47	159	2·38	+7·25	11·05
23	95	30	125	3·17	—1·25	9·79
24	77	26	103	2·96	+0·25	8·89
25	107	38	145	2·82	+1·75	10·51
26	96	33	129	2·91	+0·75	9·95
27	97	25	122	3·88	—5·50	9·68
28	74	18	92	4·11	—5·00	8·40
29	43	8	51	5·38	—4·75	6·25
30	97	25	122	3·88	—5·50	9·68
31	63	21	84	3·00	0·00	8·03
32	57	18	73	3·17	—0·25	7·48
33	79	20	99	3·95	—4·75	8·72
34	35	19	54	1·84	+5·50	6·44
35	95	37	132	2·57	+4·00	10·07
36	64	21	85	3·05	—0·25	8·08
37	4	2	6	2·00	+0·50	2·15
38	12	3	15	4·00	—0·75	3·39
39	9	1	10	9·00	—1·50	2·77
40			No setting			
41	5	3	8	1·67	+1·00	2·46
42	22	5	27	4·40	—1·75	4·56
43	1	1	2	1·00	+0·50	1·24
44	1	0	1	0·00	—0·25	0·88
45			No setting			
46	2	0	2	0·00	—0·50	1·24
47	4	2	6	2·00	+0·50	2·15
48			No setting			
49	5	2	7	2·50	+0·25	2·33
50	6	3	9	2·00	+0·75	2·63
51	9	4	13	2·25	+0·75	3·16
52	23	6	29	3·83	—1·25	4·72
53	43	11	54	3·91	—2·50	6·44
54	43	17	60	2·53	+2·00	6·79

Here the deviation, being greater than 3 P. E., is significant.

$$\chi^2 = 32{\cdot}29.$$

$$\sqrt{2\chi^2} - \sqrt{2n-1} = -2{\cdot}01.$$ The fit is good.

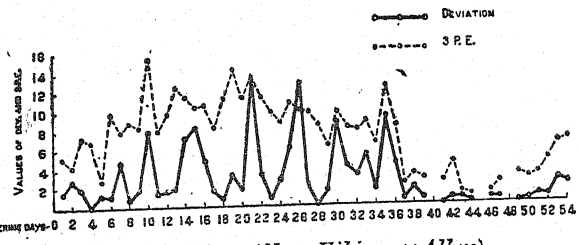

Fig. 2. Stem-colour (*New Hibiscus*×*Albus*).

As before, it could be safely concluded that neither the age of the F_1 plant nor the season has any influence on the F_2 segregation.

The frequencies of the two phenotypes for the entire flowering period are as follows :—

	Red stem (various grades)	Green stem	Total
Total observed	3357	1117	4,474
Total expected on 3 : 1 basis	3355·5	1118·5	4,474
Deviation	1·5	1·5	

$$\frac{\text{Dev,}}{\text{P. E.}} = \frac{1{\cdot}50}{1953} = 0{\cdot}08$$

The fit is, therefore, excellent.

The 'fit' to a 3 : 1 ratio was good for the population of each F_1 plant taken individually.

The "fit" for the frequencies during the whole flowering period was tested and was good.

New Hibiscus × *Ruber*
Leaf-lobe

The *New Hibiscus* has a narrow leaf-lobe and *Ruber*, a broad leaf-lobe. The F_1 had a narrow leaf-lobe and in F_2 a ratio of 3 narrow-lobed plants : 1 broad-lobed plant is expected on theory in the progeny of each successive day. The actual results are shown in the following table :—

TABLE III

Showing the daily frequencies of the two phenotypes, their ratios, total population, deviation between observed and theoretical frequency of the recessive, and 3 P. E.

New Hibiscus × *Ruber*—F_2
Leaf-lobe

Flowering day	Narrow lobe	Broad lobe	Total	Ratio	Deviation	3 P. E.
1	19	11	30	1·73	+3·50	4·80
2	27	4	31	6·75	—3·75	4·88
3	52	20	72	2·60	+2·00	7·44
4	36	15	51	2·40	+2·25	6·25
5	75	24	99	3·12	—0·75	8·72
6	125	46	171	2·72	+3·25	11·47

TABLE III—*contd.*

Flowering day	Narrow lobe	Broad lobe	Total	Ratio	Deviation	3 P. E.
7	110	43	153	2·56	+4·75	10·84
8	94	33	127	2·85	+1·25	9·9
9	38	16	54	2·37	+2·50	6·43
10	144	38	182	3·79	—7·50	11·82
11	43	21	64	2·05	+5·00	7·01
12	43	18	61	2·39	+2·75	6·84
13	43	13	56	3·31	—1·00	6·55
14	105	38	143	2·76	+2·25	10·48
15	57	18	75	3·17	—0·75	7·58
16	50	17	67	2·94	0·25	7.17
17	108	28	136	3·86	—6·00	10·24
18	64	23	87	2·78	+1·25	8.17
19	202	68	270	2·97	+0·50	14·36
20	150	59	209	2·54	+6·75	12·70
21	101	28	129	3·61	—4·25	9·95
22	81	38	119	2·13	+8·25	9·63
23	58	19	77	3·05	—0·25	7·68
24	89	32	121	2·78	+1·75	9·63
25	88	27	115	3·26	—1·75	9·37
26	126	38	164	3·32	—3·00	11·21
*27	119	56	175	2·13	+12·25	11·56
28	57	20	77	2·85	+0·75	7·68
29	51	20	71	2·55	+2·25	7·38
30	56	16	72	3·50	—2·00	7·44
31	36	16	52	2·25	+3·00	6·31
32	42	13	55	3·23	—0·75	6·50
33	53	14	67	3·79	—2·75	7·17
34	31	9	40	3·44	—1·00	5·53
35	49	9	58	5·42	—5·50	6·67
36	30	17	47	1·76	+5·25	6·01
37	13	4	17	3·25	—0·25	3·61
38	5	2	7	2·50	+0·25	2·32
39	1	2	3	0·50	—1·25	1·52
40	6	2	8	3·00	0·00	2·88
41	2	1	3	2·00	+0·25	1·52
42	6	2	8	3·00	0·00	2·48
43	5	1	6	5·00	—0·50	2·15
44	1	0	1	0·00	—0·25	0·88
45	1	1	2	1·00	+0·50	1·24
46			No setting			
47			,, ,,			
48			,, ,,			
49						
50	0	1	,, ,, 1	0·00	+0·75	0·88
51			No setting			

*Here the deviation, being greater than 3 P. E., is significant.

TABLE III—*concld.*

Flowering day	Narrow lobe	Broad lobe	Total	Ratio	Deviation	3 P. E.
52	21	7	28	3·00	0·00	4·63
53	42	15	57	2·80	+0·75	6·61
54	15	4	19	3·75	—0·75	3·82

$\chi^2 = 39{\cdot}08$

$\sqrt{2\chi^2} - \sqrt{2n-1} = -1{\cdot}01$. The fit is good.

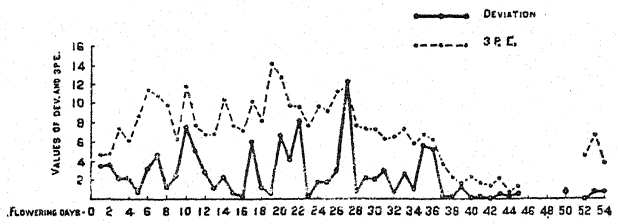

FIG. 3. Leaf-lobe (*New Hibiscus* × *Ruber*).

The table and Fig. 3 do not show seasonal or age (of F_1) effect on the segregations, the deviation between the observed and theoretical frequencies being significant only on the 27th day. Thus the conclusion as in the previous cases is that the F_2 ratios are not affected by the time of selfing of the F_1 plants, nor by any other factor so far as this character, *viz.*, leaf-lobe, in *Hibiscus* is concerned.

The F_2 phenotypic frequencies for the entire flowering period are as follows :—

	Narrow-lobed plants	Broad-lobed plants	Total
Total observed	2770·00	967·00	3,737
Total expected on 3 : 1 basis	2802·75	934·25	3,737
Deviation	32·75	32·75	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{32{\cdot}75}{17{\cdot}73} = 1{\cdot}85$$

The fit, therefore, is good.

Out of the five F_2 populations from the five F_1 plants "fits" to a 3 : 1 ratio were good in four cases and bad in one.

The "fit" for the frequencies during the whole flowering period was tested and was good.

Stem-colour.—*New Hibiscus* has a green stem with red patches in the leaf-axil and below it. *Ruber* has a red stem. In F_1, the stem was red. In F_2, a ratio of 3 red-stemmed plants (like *Ruber* and F_1) : 1 green-stemmed with patches is

theoretically expected. The actual observed results for each flowering day are given in the following table :—

TABLE IV

Showing the daily frequencies of the two phenotypes, their ratios, total population, deviation between observed and theoretical frequency of the recessive and 3 P. E.

New Hibiscus × *Ruber* F_2

Stem-colour

Flowering day	Red stem	Green stem	Total	Ratio	Deviation	3 P. E.
1	22	7	29	3·14	—0·25	4·72
2	25	6	31	4·17	—1·75	4·88
3	55	15	70	3·33	—2·50	7·33
*4	30	21	51	1·43	+8·25	6·25
5	81	18	99	4·50	—6·75	8·72
6	122	50	172	2·44	+7·00	11·49
7	119	36	153	3·31	—2·25	10·84
8	94	33	127	2·85	+1·25	9·90
9	35	19	54	1·84	+5·50	6·43
10	141	47	188	3·20	0·00	12·00
11	50	14	64	3·57	—2·00	7·01
12	41	20	61	2·05	+4·75	6·84
13	35	15	50	2·33	+2·50	6·19
14	112	32	144	3·75	—4·00	10·51
15	58	19	77	3·05	—0·25	7·68
16	57	12	69	4·75	—5·25	7·28
17	105	32	137	3·28	—2·25	10·25
18	62	23	85	2·70	+1·75	8·08
19	189	82	271	2·31	+14·25	14·45
20	156	53	209	2·95	+0·75	12·70
*21	75	39	114	1·92	+10·50	9·37
22	95	35	130	2·71	+2·50	9·98
23	61	16	77	3·81	—3·25	7·68
24	83	38	121	2·18	+7·75	9·63
25	82	33	115	2·58	+4·25	9·37
26	115	49	164	2·35	+8·00	11·21
*27	115	64	179	1·79	+19·25	11·74
28	52	25	77	2·08	+5·75	7·68
29	53	19	72	2·79	+1·00	7·44
30	57	15	72	3·80	—3·00	7·44
31	40	12	52	3·33	—1·00	6·31
32	40	15	55	2·67	+1·25	6·50
33	52	16	68	3·25	—1·00	7·23
34	28	13	41	2·15	+2·75	5·61
35	47	12	59	3·92	—2·75	6·73
36	30	15	45	2·00	+3·75	5·88
37	17	5	22	3·40	—0·50	4·11
38	4	3	7	1·33	+1·25	2 32
39	1	2	3	0·50	+1·25	1·52

TABLE IV—*contd.*

Flowering day	Red stem	Green stem	Total	Ratio	Deviation	3 P. E.
40	6	2	8	3·00	0·00	2·48
41	2	1	3	2·00	+0·25	1·52
42	5	3	8	1·67	+1·00	2·48
43	5	1	6	5·00	—0·50	2·15
44	1	0	1	0·00	—0·25	0·88
45	2	0	2	0·00	—0·50	1·24
46			No setting			
47			,, ,,			
48			,, ,,			
49			,, ,,			
50	1	0	1	0·00	—0·25	0·88
51			No setting			
*52	15	13	28	1·15	+6·00	4·63
53	39	20	59	1·95	+5·25	6·73
54	15	4	19	3.75	—0·75	3·82

*Here the deviations, being greater than 3 P. E., are significant.

$\chi^2 = 70 \cdot 594$.

$\sqrt{2\chi^2} - \sqrt{2n-1} = 1 \cdot 95$. The fit is good.

Table IV shows the following facts :—

(1) There seems to be a tendency for the dominants to occur in excess during the first seventeen days with, however, six exceptions. During the next twelve days, on the other hand, there is a tendency for the recessives to be in excess excepting on one day (23rd), and two of the deviations are significant. The later deviations, positive and negative, are fairly random.

(2) During the whole flowering period only four deviations, those on the 4th, 21st, 27th, and 52nd days are significant. In a large majority of cases, *i.e.*, 92 per cent, however, the deviations are due to chance.

Figure 4 shows some of the above points very clearly. Though there is a slight tendency towards a periodic fall and rise in the number of the recessives, yet most of the deviations not being statistically significant, the periodicity may be due to chance, and it cannot be safely concluded that either the age of the F_1 plant, or any other factor has any influence on the F_2 segregation.

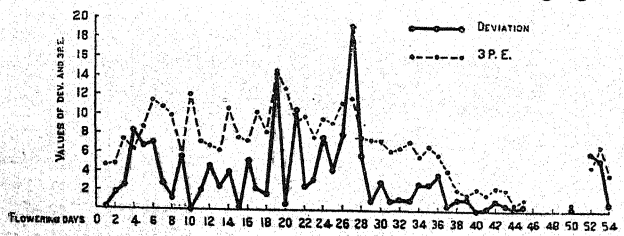

Fig. 4. Stem-colour (*New Hibiscus* × *Ruber*).

The total population for the full flowering period shows the following phenotypic frequencies :—

—	Stem red	Stem green with red patches	Total
Total observed	2,727·00	1,024·00	3,751
Total expected on 3 : 1 basis	2,813·25	937·75	3,751
Deviation	86·25	86·25	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{86{\cdot}25}{17{\cdot}76} = 4{\cdot}86.$$ The fit is bad.

The excess in the recessives is responsible for a bad fit, but it cannot be accounted for. In one, out of the five different F_2 populations, the " fit " to a 3 : 1 ratio was bad.

The " fit " for the frequencies during the whole flowering period was tested and was good.

IV. Second series of experiments

Two crosses were studied when the experiment was repeated in the year 1934-35. One was *Albus* × *Ruber* with a F_2 population of about 12,600 and the other *Ruber* × *New Hibiscus* with a F_2 population of about 22,000. The latter was a repetition of the previous year's work, the former a new cross, in which both parents possessed a broad leaf-lobe, and stem-colour was the only factor considered. The crosses were made in the year 1932-33.

The hybrid seeds were sown in June, 1933. The germination was uniform in all the F_1 seed. There were six F_1 plants of the former cross and seven of the latter. All the plants began to flower practically at the same time. In the cross, *Albus* × *Ruber*, flowering commenced in one plant on November 2, 1933, and by November 6, 1933, all were in flower. In the other cross, *Ruber* × *New Hibiscus*, flowering commenced on November 3rd, 1933, in one plant, and by November 5th, 1935, all of them were in flower. Selfing was begun from the very commencement of flowering until practically the end of the flowering period in each cross. In the former cross, plants were selfed for about thirty successive days, and in the latter for about sixty succesive days. The flowers towards the end of the season, however, either did not set or only produced very few seeds, and on this account seeds of the first twenty-five days in the former cross and of forty-three days in the latter were sown to grow the F_2 populations.

The methods of sowing, cultivation, selfing, etc., were the same as in the previous year with the difference that nearly all the flowers on each F_1 plant were selfed to ensure large F_2 populations.

The selfed seeds of the F_1 plants were sown on June 20th, 1934. The germination was quite satisfactory unlike that in the previous year, making available for study a population of about 12,600 in the cross *Albus* × *Ruber* and about 22,000 in the cross, *New Hibiscus* × *Ruber*.

The observations were commenced when the plants were about four weeks old. The differences between the different phenotypes were quite clear-cut, and hence there was no possibility of mistaking one phenotype for the other.

The statements of daily segregations, etc., have not been presented in this series of experiments. The results will, however, be clear from the illustrations.

Experimental results

Albus × *Ruber*

Stem-colour.—Albus has a green stem and *Ruber* has red. The F_1 had red stem and in F_2, a ratio of 3 red stems : 1 green stem is theoretically expected [Howard and Howard, 1924] in each day's population. The expectation is realized as will be evident from the results illustrated in Fig. 5.

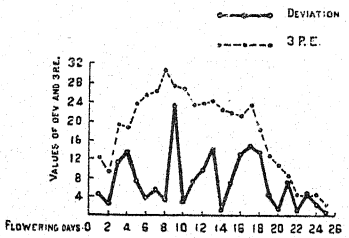

Fig. 5. Stem-colour (*Albus* × *Ruber*).

The figure shows that the ratio of dominants to recessives varies from day to day without any periodicity. It also shows that only one deviation, that on the 23rd, is significant and that in ninety-six per cent cases, the deviations are not significant, being less than 3 P. E.

The following results are obtained when the frequencies for all the flowering days are added up :—

——	Red stem	Green stem	Total
Total observed	9,390·00	3,251·00	12,641
Total expected on 3 : 1 basis . . .	9,480·75	3,160·25	12,641
Deviation	90·75	90·75	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{90 \cdot 75}{32 \cdot 83} = 2 \cdot 7.$$ The fit is fairly good.

One F_2 population out of the five has given a bad " fit " to a 3 : 1 ratio.

From the above results it may be concluded that the F_2 results are not influenced by age of the plant or environmental factors.

New Hibiscus × *Ruber*

Leaf-lobe.—The results of the daily segregations of the two phenotypes, narrow or broad leaf-lobe, are shown in Fig. 6. The figure shows that during the first five days the dominants are in excess, while during the next seven days the increase or decrease in them is fairly random. The next twenty days, except one, show an excess of dominants. Then there is again a random occurrence of excess and deficiency in them during the last six days. As will be evident from the figure the deviations are, however, not significant, except on three days, *viz.*, 10th, 12th, and 16th, *i.e.*, in about ninety-three per cent cases.

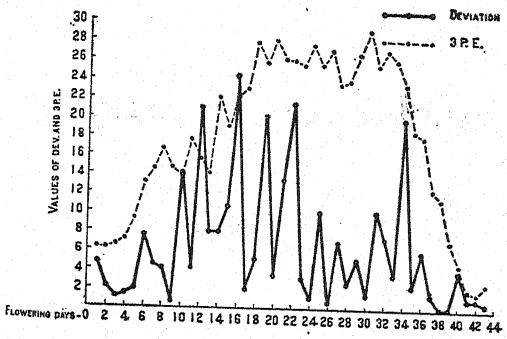

Fig. 6. Leaf-lobe (*New Hibiscus* × *Ruber*).

As before, it may again be concluded that the F_2 ratios are not affected by the age of the F_1 plant or by any other factor.

The combined frequencies for the whole flowering period, which give a good "fit" to a 3 : 1 ratio, are as follows :—

——	Narrow leaf-lobed plants	Broad leaf-lobed plants	Total
Total observed	16,686	5,398	22,084
Total expected on 3 : 1 basis . .	16,563	5,521	22,084
Deviation	123	123	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{123}{43 \cdot 39} = 2 \cdot 83.$$ The fit is fairly good.

All the five F_2 populations have each given a good "fit" to a 3 : 1 ratio individually.

The "fit" for the frequencies during the whole flowering period was tested and was good.

Stem-colour.—The day-to-day results of the segregations of the two phenotypes, red and green, are illustrated in Fig. 7, from which it will be evident that the daily deviations, positive and negative, from the expected results, occur fairly at random and that except on five days, *viz.*, 10th, 15th, 19th, 30th and 38th they are not significant, being less than three times the Probable Error. That is in only twelve per cent cases the deviations are significant and in eighty-eight per cent cases they are due to chance alone. So, as before, it could be concluded that the age of the F_1 plant, or any other factor does not interfere with the F_2 ratio.

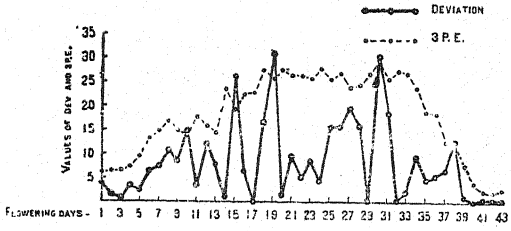

Fig. 7. Stem-colour (*New Hibiscus* × *Ruber*).

The combined frequencies of the two phenotypes for all the flowering days are as follows :—

——	Red stem	Green stem	Total
Total observed	16,396	5,628	22,024
Total expected on 3 : 1 basis . .	16,518	5,506	22,024
Deviation	122	122	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{122}{43 \cdot 33} = 2 \cdot 82.$$ The fit, therefore, is fairly good.

All the five F_2 populations have given good " fits " to a 3 : 1 ratio individually.

The " fit " for the frequencies during the whole flowering period was tested and was good.

V. Discussion

The present investigation deals with two different, distinct characters, one, *viz.*, stem-colour in three different crosses and the other, *viz.*, leaf-lobe in two different crosses, the investigations for one cross having been repeated with a very large population. The results of any of the crosses in either year do not show any significant deviation, periodic or otherwise, in the F_2 ratios.

Considering the first year's results of our investigation, we find that in the cross, *New Hibiscus* × *Albus*, the observed results in the case of the character leaf-lobe significantly deviate only on one day and that all other deviations are not statistically significant. There is no indication of the deviations having a periodic trend. In the stem colour character also the observed results significantly deviate from theoretical ones only on one day during the whole reproductive period of the

plant. Moreover, in the rest of the deviations, which are not significant, there is not the slightest indication of periodicity.

With respect to the other cross, *New Hibiscus* × *Ruber*, the observed results for the leaf-lobe character significantly deviate only once during the entire reproductive period of the mother plant. There is absolutely no indication of any of the deviations being periodic. The results for the other character, *viz.*, stem-colour, show that all the deviations from the expected results, except four, are within the limits of chance errors. There is, however, a slight tendency for the dominants to be in slight excess in the beginning and to be slightly deficient thereafter for a few days. But as judged by the criterion, *viz.*, " goodness of fit ", most of the deviations are not statistically significant, and the slight periodicity, if it be so called, may be taken as due to chance. If one suspects periodic rise or fall in the number of the dominants or recessives in this cross and for the character, leaf-lobe, then it may be questioned as to why this phenomenon did not manifest itself in the second series of experiments when this cross was duplicated with a larger population. The failure of the periodicity to make its appearance in the cross when repeated supports the conclusion that the above periodicity is due to chance.

Considering now the results of the second series of experiments, and taking first the cross *Albus* × *Ruber*, it is seen that only on one day do the observed results deviate statistically from the theoretical. Also there is no indication of the deviations being periodic.

In the other cross, *New Hibiscus* × *Ruber*, the results for the leaf-lobe character show that only on three days out of forty-three do the observed results significantly deviate from the expected ones. No tendency for a periodic rise or fall in the number of either of the phenotypes is indicated. For the stem-colour character, the deviations between the observed and expected results are significant only in four cases, and indication of any periodicity in the results is absent.

VI. Summary and conclusion

The mass of evidence set out in the foregoing paper shows clearly that in the case of *Hibiscus sabdariffa* L., the segregation in the F_2 generation of the characters, leaf-lobe and stem-colour, are not influenced by the time at which fertilisation occurs in the heterozygous parent plants.

Selfing of the F_1 generation may, therefore, be carried out at any stage during the flowering period without danger of disturbing the normal Mendelian ratios.

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AN ILLUSTRATION OF THE USE OF THE χ^2 -TECHNIQUE ANALYSIS ON METEOROLOGICAL DATA

BY

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The χ^2-technique of analysis is frequently employed in statistical investigations on biological data. The technique was first given by R. A. Fisher in connection with the problem of estimating the density of bacterial populations in sugar refinery products. It has since been applied in some form or other to numerous other problems of practical value. It is intended here to exhibit its application to yet another problem arising in Agricultural Meteorology and to put it in a suitable form so as to be immediately available for application in practice.

The data* which form the subject of study in this paper relate to the numbers of counts of nuclei made with the Aitken portable counter in the open air at Kew Observatory. In Table I are given the results of 240 such counts, which were completed in about an hour, a short pause being made at every five minutes in order to write down the figures.

A column contains the results of twenty consecutive counts which occupied about five minutes. The object is to ascertain whether a genuine change was brought about in the nucleus-content of the air during the hour of observation or else the fluctuations in counts were simply due to errors of random sampling.

Now it has been shown by F. J. Scrase that if the nuclei in air are distributed at random, then the probability of occurrence of a count of a given value is given by the term of the Poisson Series. Consequently regarding the data in Table I as having been drawn in random sampling from one or more Poisson distributions, the enquiry reduces to direct application of χ^2-tests to these data as described by Fisher.

* These data have been discussed by F. J. Scrase in his paper 'On the Sampling Errors of the Aitken Nucleus Counter,' *Jour. Roy. Met. Society*, London. Vol. LXI, 1935. These were kindly sent to me, at my request, for further analysis.

TABLE I

Kew Observatory : Nucleus counts made on December 29th, 1933

	1	2	3	4	5	6	7	8	9	10	11	12	
G. M. T.	11.47½	11.52½	11.57½	12.02½	12.07½	12.12½	12.17½	12.22½	12.27½	12.32½	12.37½	12.42½	
1	6	8	4	4	3	8	5	8	7	4	8	6	
2	3	11	8	5	2	8	10	6	4	11	1	10	
3	4	6	6	7	3	4	6	4	4	8	4	11	
4	6	6	9	8	6	9	6	5	8	6	1	3	
5	7	11	7	3	4	15	5	7	2	6	5	3	
6	4	7	9	10	8	16	7	7	5	4	6	2	
7	3	8	3	13	9	8	3	7	3	7	6	1	
8	10	6	3	12	7	11	6	5	2	8	0	1	
9	4	3	9	11	12	7	4	8	5	6	7	2	
10	6	6	8	5	4	14	6	3	4	6	4	3	
11	5	9	6	8	7	17	7	11	8	7	5	6	
12	7	8	8	7	2	7	4	5	6	7	5	1	
13	4	6	11	7	3	5	2	5	3	6	7	6	
14	5	5	15	3	1	6	2	6	4	6	6	4	
15	10	10	8	5	5	6	4	5	5	7	8	3	
16	8	11	6	11	6	10	7	4	6	9	14	4	
17	6	4	5	8	4	5	4	5	6	1	4	4	
18	10	7	7	7	6	4	4	4	5	11	6	5	
19	6	8	6	4	2	4	4	5	4	5	2	5	
20	8	11	2	6	2	12	4	5	7	2	8	3	
Totals C_i	122	151	140	144	98	176	100	115	98	127	107	83	
χ^2	15.4	14.4	24.3	23.2	26.9	36.0	14.0	11.1	12.2	18.7	34.9	33.4	($\chi^2_{5\%} = 30.1$).

Unlike the 'Analysis of Variance' Technique for Normal Variation, the χ^2-technique does not appear to have been put in a tabular form. In view of its frequent application to biological data, it will be well worth the while to give in a tabular form the χ^2-tests, which are appropriate for the analysis of data coming from the Poisson and binomial populations and then to discuss the data in Table I in the light of these tests.

Suppose we have a type of data arranged as follows in a double classification.

$$\begin{array}{cccc} x_{11} & x_{21} & x_{t1} & x_{k1} \\ x_{12} & x_{22} & x_{t2} & x_{k2} \\ .. & .. & .. & .. \\ .. & .. & .. & .. \\ x_{1i} & x_{2i} & x_{ti} & x_{ki} \\ .. & .. & .. & .. \\ x_{1n} & x_{2n} & x_{tn} & x_{kn} \end{array} \qquad (1)$$

where x_{ti} represents the number of occurrences counted in a fixed time or space interval and the chance that the variable in the t_{th} column and the i_{th} row takes the value x_{ti} is given by

$$\epsilon^{-m_{ti}} \frac{(m_{ti})^{x_{ti}}}{x_{ti}!} \qquad (2)$$

The problems for consideration before us are whether

(*a*) the variation within columns is no more than might be expected through chance causes

(*b*) the variation between columns is within the limits of errors of random sampling, assuming that the variation within columns is ascribable to pure chance causes

(*c*) the whole set of records could be combined together without loss of homogeneity.

Analogous problems arise in the case of binomial population. The data such as these in Table I may now arise in a variety of ways. Thus, for example,

the n values of x in the t^{th} column may represent the number of infested barley ears in n samples of, say, 100, drawn from the t^{th} plot and k will represent the number of plots; and the problems for consideration would then be whether the material is homogeneous within a plot, whether the proportion of infestation varies considerably from plot to plot and so on.

The form of χ^2-technique is given in Table II, where in the case of the Poisson Series

$\bar{x}_{t.}$ represents the mean of the t^{th} column

$\bar{x}_{..}$ represents the mean of the total set of observations

and in the case of the Binomial series—

$p_{t.}$ represents the proportion of infested ears on the t^{th} plot

$p_{..}$ is the proportion of infested ears on all the plots

$q_{t.} = 1 - p_{t.}$ and $q_{..} = 1 - p_{..}$

TABLE II

Variation	Sums of squares	χ^2 for Poisson	χ^2 for binomial	f= degrees of freedom
Within classes	$\sum_{t=1}^{k}\sum_{i=1}^{n}(x_{ti}-\bar{x}_{t.})^2$	$\sum_{t=1}^{k}\left\{\frac{\sum_{i=1}^{n}(x_{ti}-\bar{x}_{t.})^2}{\bar{x}_{t.}}\right\}$	$\sum_{t=1}^{k}\left\{\frac{\sum_{i=1}^{n}(x_{ti}-\bar{x}_{t.})^2}{\bar{x}_{t.}q_{t.}}\right\}$	$k(n-1)$
Between classes	$n\sum_{t=1}^{k}(\bar{x}_{t.}-\bar{x}_{..})^2$	$\frac{n\sum_{t=1}^{k}(x_{t.}-x_{..})^2}{\bar{x}_{..})}$	$\frac{n\sum_{t=1}^{k}(\bar{x}_{t.}-\bar{x}_{..})^2}{\bar{x}_{..})\,q_{..}}$	$k-1$
Total	$\sum_{t=1}^{k}\sum_{i=1}^{n}(x_{ti}-\bar{x}_{..})^2$	$\frac{\sum_{t=1}^{k}\sum_{i=1}^{n}(x_{ti}-\bar{x}_{..})^2}{\bar{x}_{..}}$	$\frac{\sum_{t=1}^{k}\sum_{i=1}^{n}(x_{ti}-\bar{x}_{..})^2}{\bar{x}_{..}}$	$kn-1$

The table is similar to that used in the process of the Analysis of Variance for Normal Variation, except for column (3) where the mean square in the Analysis of Variance is now replaced by χ^2. The mean square is obtained by dividing the sum of squares of deviations by the corresponding degrees of freedom entered in column (5) ; the χ^2 is obtained by dividing the sum of squares of deviations in a class by the variance of the variate in the class. The computation involved in obtaining a χ^2 is extremely simple. If $x_1 \ldots . x$ are a sample from a Poisson Series

$$\chi^2 = \frac{\sum_{i=1}^{n} (x - \bar{x})^2}{\bar{x}}$$

$$= \frac{n \sum_{i=1}^{n} x_i^2}{T} - T \qquad . \quad . \qquad (3)$$

where

$$T = n\bar{x} = \sum_{i=1}^{n} x_i$$

a form which is extremely convenient for computation, noting especially that $x_1 \ldots x_n$ are all integers. For the binomial we have similarly

$$\chi^2 = \frac{1}{q} \left\{ \frac{n \sum_{i=1}^{n} x_i^2}{T} - T \right\} \qquad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

The form of analysis given above is of general application and deals simply with variation within and between classes. Sometimes, however, it may be required to subdivide the total contribution to χ^2 into its components corresponding to desired groups of (or single) degrees of freedom. The process is of interest from another point of view ; namely of locating the main discrepancy in case a significant value of χ^2 is obtained, which may in fact be due to some individual degrees of freedom corresponding to real effects which may be of importance to our enquiry.

This process of subdivision of χ^2 into its components is fully developed by Fisher [1934] and no space will, therefore, be devoted here for its discussion. In its simple form it will be employed in the analysis of data in Table I, which is discussed below.

Following the entries in Table II, the values of the observed χ^2 for variation within and between columns are entered in column 2 of Table III. The corresponding five per cent values are given in column (3). It will be noted that the value of χ^2 for variation within columns is the total of twelve values of χ^2, entered at the bottom of each column in Table I.

TABLE III

Variation	d. f.	χ^2	χ^2 5 per cent	
Within columns	228	264·4	263·9	
Between columns	11	65·9	19·7	

Judged by the five per cent level of significance, the values of χ^2 for variation within and between columns are both significant, although the former is barely so. The values of χ^2 for columns 6, 11 and 12 are mainly responsible for the fat total of 264·4, which represents the χ^2 for variation within columns. With their exception, the variation may effectively be regarded as having arisen due to errors of random sampling.

The significance of χ^2 for variation between columns leaves no doubt whatever on the point that a genuine change had in fact been brought about in the nucleus content of the air. It may, however, be asked whether the significance of the χ^2 value might not solely be due to exceptionally high and low totals for columns 6 and 12. Indeed the χ^2's obtained for these columns suggest that a significant variation in the composition of the air was brought about at the time when observations in these columns were being taken. The contribution to χ^2 for variation between columns can thus be divided into two components χ^2_1 and χ^2_2 the first made by all columns except 6th and 12th and consequently based on nine degrees of freedom, while the latter based on the remaining two degrees of freedom which are orthogonal to the first nine.

Denote the totals for columns in Table I by $C_1 \ldots . C_{12}$. On the hypothesis of equal expectation in all the 12 columns, χ^2_1 is obtained by dividing the sum of squares of deviations of $C_1 \ldots \ldots C_5, C_7 \ldots \ldots C_{11}$ by the variance of Ci which is 1461/12= 121·75 ; while χ^2_2 is obtained as the sum of χ^2's corresponding to degrees of freedom given by

$$5\,(C_6 + C_{12}) - (C_1 + \ldots . + C_5 + C_7 + \ldots + C_{11})$$

and $C_6 - C_{12}$ which are 1·2 and 35·5 respectively.

Thus we have

$\chi^2_1 = 29{\cdot}2$ and $\chi^2_2 = 36{\cdot}7$,

their total being 65·9, agreeing with the value of χ^2 for variation between columns as it should.

The five per cent values of χ^2 based on nine and two degrees of freedom respectively are 16·9 and 6·0. Both χ^2_1 and χ^2_2 are thus seen to be far above their five per cent values, suggesting that the change in the content of the air was not entirely due to two sudden disturbances but was probably brought about gradually.

It is remarkable to note that of the contribution to χ^2_2 made by the two degrees of freedom, namely

$$5\,(C_6 + C_{12}) - (C_1 + \ldots + C_5 + C_7 + \ldots + C_{11})$$

and $C_6 - C_{12}$

the contribution made by the first is only 1·2 while that made by the latter is 35·5, showing how more than half the contribution to total χ^2 for variation between columns is accounted for by a single degree of freedom corresponding to difference between the total number of counts observed from $12{\cdot}12\frac{1}{2}$ to $12{\cdot}17\frac{1}{2}$ and $12{\cdot}42\frac{1}{2}$ to $12{\cdot}47\frac{1}{2}$ respectively. That this contribution made by the contrast $C_6 - C_{12}$ is so high as 35·5 should not, however, be a matter for surprise, since the two values chosen are such that they yield the largest apparent difference between any two values of the set. It is clearly possible that the difference between the highest and lowest values in the case of a fairly large sample will appear significant even though the material forming the sample is undifferentiated and homogeneous ; much more so when the material is known to be heterogeneous as it probably is in our example. The natural remedy to judge the true significance of the selected contrast is, therefore, to raise the usual level of significance in the inverse ratio of the total number of ways in which the values appearing in the contrast could equally be chosen from among the whole set. Since 1 in 20 is the level of significance employed in this paper we should expect that the level of significance for the special contrast should be 1/1320. The probability that the value of χ^2 for a single degree of freedom should be as large as 35·5, which is the value of χ^2 for the contrast $C_6 - C_{12}$, is found from the tables to be smaller than 1/1320, so that in spite of selection, the contrast $C_6 - C_{12}$ is statistically significant judged by the new level of significance thus obtained.

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STUDIES IN SORGHUM—THE GREAT MILLET

III. ANTHER, POLLEN AND STIGMA

BY

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(With Plates LXVII & LXVIII and two text-figures)

INTRODUCTION

The anthesis and pollination of sorghum has been described in the first part of these studies [Rangaswami Ayyangar and Panduranga Rao, 1931]. In this part will be presented some studies on the anther, pollen and stigma--studies that have been in progress since 1931.

THE ANTHER

Anther-size.—Variation in anther size is a varietal character. Even when devoid of pollen, the anther sacs in this cereal do not collapse. The sacs were measured closely after emergence.

TABLE I

[*Length and diameter of anthers*

(Average of 100)

Varieties	Length of anthers in mm.	Diameter of anthers in μ
S. roxb. var. *hians*	3·5	1,086
S. durra.	3·25	1,061
S. nervosum	3·0	975
S. margaritiferum	2·75	888
Andropogon sorghum, var. *technicus*	2·75	876

The reduction in the size of the anther from the grain sorghum types to others with a lax panicle is interesting.

The common grain sorghum is *S. durra*. In the ear-heads of this as in other sorghums there are both sessile and pedicelled flowers. The sessile flowers are hermaphrodite. The pedicelled flowers are mostly abortive, but occasionally bear anthers. These two sets of anthers were measured 100 each and gave an average length of 3·25 mm. and an average width of 998 μ in each set, thus showing no differences in size consequent on sexual atrophy.

Grain sorghums are usually single-stalked. Sometimes there are tillers which give ear-heads. These ear-heads lack vigour and are always markedly loose in contrast to the compact ear-head from the main stalk. The length and breadth of anthers produced on the main head were measured and compared with those on the side shoots (100 each) and no difference was noted between them.

Pollen

The pollen grains of sorghum are smooth and spherical and contain a single germ-pore. They vary in size and are roughly parallel to anther size differences.

Variety	Size range
S. roxb. var. *hians*	43 μ-62 μ
S. cernuum	37 μ-56 μ
S. durra	
A. S. var. *technicus*	37 μ-49 μ
S. nervosum	
S. margaritiferum	

For a comparative study the pollen grains of the wild grass *Andropogon annulatus* L. were measured and gave a range of 25 μ - 37 μ. The lessening of the pollen size in the wild *Andropogon* and in the lax-headed sorghums is very interesting.

Dummy pollen

In the course of examination of innumerable pollen grains in many varieties of sorghum a peculiar kind of pollen is met with. An examination of pollen under the microscope shows constantly a few grains devoid of solid contents (Plate LXVII). In size they are comparatively small and measure 31 μ- 34 μ. They are not shrivelled but retain normal shape owing to the pressure of the sap inside. They usually plasmolyse in the culture medium and do not germinate. These peculiar pollen grains have been provisionally named " dummy pollen ". [Rangaswami Ayyangar and Panduranga Rao, 1935]. Pollen studies have not, so far as we are aware, recorded non-germinating pollen of this peculiar type. Similar pollen has been met with in the allied wild grasses, *Andropogon annulatus* and *Andropogon pertusus* L.

DUMMY POLLEN

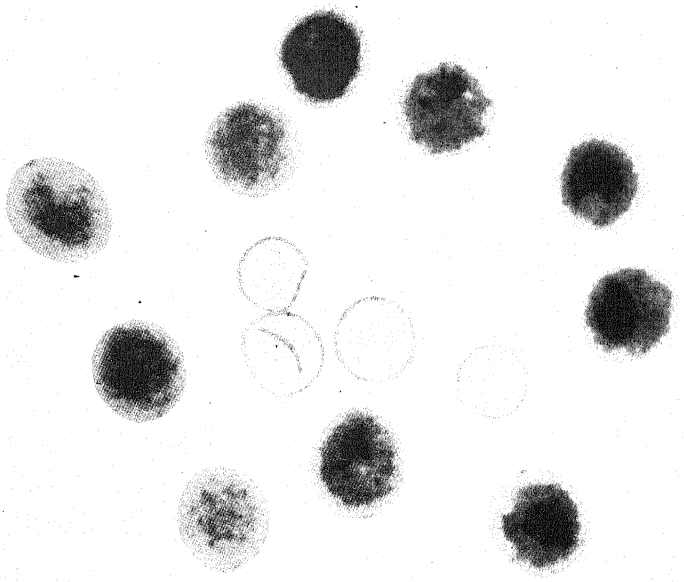


FIG. 1. *Pennisetum typhoides* (×200).

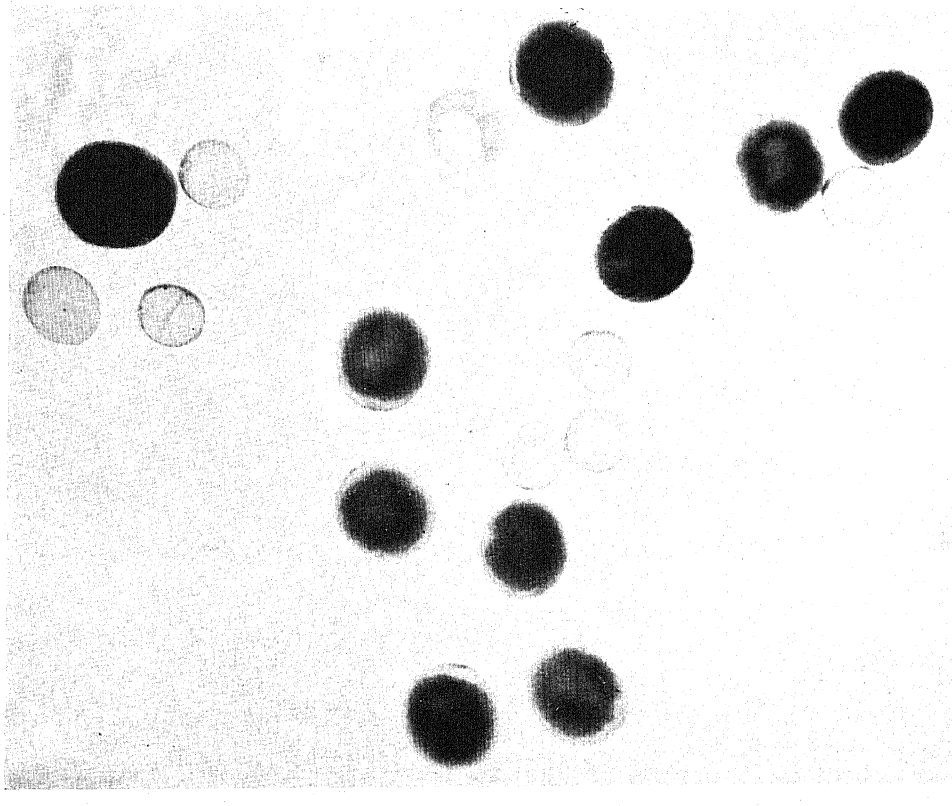


FIG. 2. *Sorghum durra* (×200).

This dummy pollen and the details of its incidence formed the subject of intensive studies. The occurrence of this peculiar pollen varies in the different varieties.

TABLE II

Distribution of dummy pollen in sorghum varieties (from anthers in the middle of the ear-head)

Varieties	Total number of normal grains	Number of dummy pollen grains	Percentage of dummy pollen grains
Rhodesian wild sorghum	4,005	624	13·5
A. S. var. *technicus*	916	113	12·3
S. papyrascens	6,327	615	9·7
S. nervosum	2,115	188	8·8
S. melaleucum	9,757	671	6·9
S. roxb. var. *hians*	1,683	63	3·9
S. durra	2,786	92	3·3
S. cernuum	1,361	32	2·3
S. margaritiferum	1,795	8	0·4
Andropogon annulatus	2,091	111	5·3

It will be seen that the higher types of cultivated varieties have a considerably lower percentage of these dummy grains.

The incidence of 'dummy pollen' shows variations within the ear-head. Several anthers were taken at random from the top, middle and bottom regions of the panicle. Their examination shows that 'dummy pollen' is slightly less in incidence in flowers at the base of the ear-head.

The incidence of the 'dummy pollen' in the unisexual flowers showed no appreciable difference in this respect. It is remarkable that this unisexual condition has not affected either the size or the nature of the pollen population in these anthers.

The occurrence of this kind of 'dummy pollen' suggested an examination of pollen grains in other millets and it was noticed that no such 'dummy pollen' could be seen in *Setaria italica, Eleusine coracana* and the *Panicum* millets. It was met with only in *Pennisetum typhoideum* Rich. (a millet of African origin) which, like sorghum, has both hermaphrodite and antheriferous flowers. In this millet this occurrence was noticed in a family in which there was considerable non-dehiscence of anthers. An examination of nearly 5,000 pollen grains in each of the dehisced and undehisced anthers showed the presence of about nine per cent of 'dummy pollen' in the former and less than one per cent in the latter. This led to a search for similar non-dehiscence in sorghum and in one instance this was met with. In this case, over 10,000 pollen grains in each of the dehisced and undehisced anthers gave about seven per cent of 'dummy pollen' in the former and only 0·6 per cent in the latter. No defects in the anther sacs were traceable. In these two millets the bulk of the flowers open during the cool hours of the night. In both of them there is a differentiation of flowers into hermaphrodite and antheriferous. The anthers of the antheriferous flowers are in the nature of a reserve to ensure pollination. A similar differentiation of functions in the pollen is, therefore, indicated with a view to ensuring the dehiscence of the anthers.

In this connection it is interesting to record the behaviour of a cross between *S. durra*, the normal grain sorghum and *S. papyrascens*, a papery-glumed sorghum in which there is erratic anthesis. In the second generation of this cross a number of plants with non-dehiscent anthers were met with. This character behaved as a simple recessive to the normal condition. An examination of the pollen grains showed that the non-dehiscence was due to the extreme paucity of 'dummy pollen' in the anthers. The above segregation is, therefore, virtually a segregation for the requisite proportion of 'dummy pollen' and the absence of the same leading to non-dehiscence and the attendant sterility (Table III.)

TABLE III

Cross Number	Character of F_1	F_2 Anthers	
		Dehisced with about 7 per cent dummy pollen	Non-dehisced with about 0·1 per cent dummy pollen
A. S. 5. CLII and CLVII.	Dehisced anthers and about 7 per cent dummy pollen . .	106	28
	Expected on 3 : 1 ratio . .	100·5	33·5
	$\chi^2 = 1·2$	P = between 0·2 and 0·3	

Pollen germination

Beyond the experiences of Patel and Patel [1929] and Stephens and Quinby [1934] there is no literature concerning experiences in the germination of pollen grains in sorghum. Patel and Patel found : "With fresh pollen from complete flowers, germination in distilled water took place after 40 minutes ; in 5 per cent glucose solution after 30 minutes ; in 10 per cent glucose solution after 25 minutes and in 15 per cent glucose solution in the few cases where germination took place at all, in about 15 minutes. In this last case, however, the pollen burst after a few minutes. In any case, the germ tube is completely extruded after 5 to 10 minutes." According to Stephens and Quinby : "Some fresh pollen germinated on a glass slide in about 20 minutes after dehiscence of the anthers. The time elapsing before the germination of other pollen grains ranged up to one hour after collection. By that time practically all the pollen had collapsed".

A number of attempts were made to germinate pollen grains of sorghum at the Millets Breeding Station, Coimbatore. Suffice it to record that the optimum concentration was found to be 41 per cent of sucrose with shred agar from 0·75 to 1·5 per cent. Experiments showed that the pollen germination was sensitive to the seasonal variation. In summer (May-June) 0·75 per cent of shred agar with 41 per cent of sucrose gave the best results, but in winter (November-December) 1·5 per cent shred agar was necessary. The germination of sorghum pollen is thus seen to be dependent on a delicate adjustment of the medium. It is so subtly sensitive to the environment that slight changes in the concentration of the medium result in an appreciable drop in the germination percentage.

TABLE IV

Optimum medium—Cultures on different days

Material — *S. durra* With 0·75 per cent agar

39 per cent sucrose			40 per cent sucrose			41 per cent sucrose			42 per cent sucrose		
Number of pollen grains on the medium		Percentage of pollen germinated	Number of pollen grains on the medium		Percentage of pollen germinated	Number of pollen grains on the medium		Percentage of pollen germinated	Number of pollen grains on the medium		Percentage of pollen germinated
Sown	Germinated		Sown	Germinated		Sown	Germinated		Sown	Germinated	
626	62	10·0	432	206	47·6	426	170	39·8	503	62	12·3
539	89	16·5	620	190	30·6	558	354	63·4	611	114	18·6
430	90	20·9	366	169	46·1	447	263	58·8	325	96	29·5
...	...	...	175	132	75·0	414	178	43·0	382	107	27·0
...	...	...	349	173	49·5	302	178	59·0	...	...	...
...	...	...	364	104	28·6	335	189	56·4	...	...	...
...	...	...	...	...	...	340	189	55·6	...	...	...
...	...	...	...	...	...	529	377	71·2	...	...	...
Average		15·1			42·2			56·6			20·8

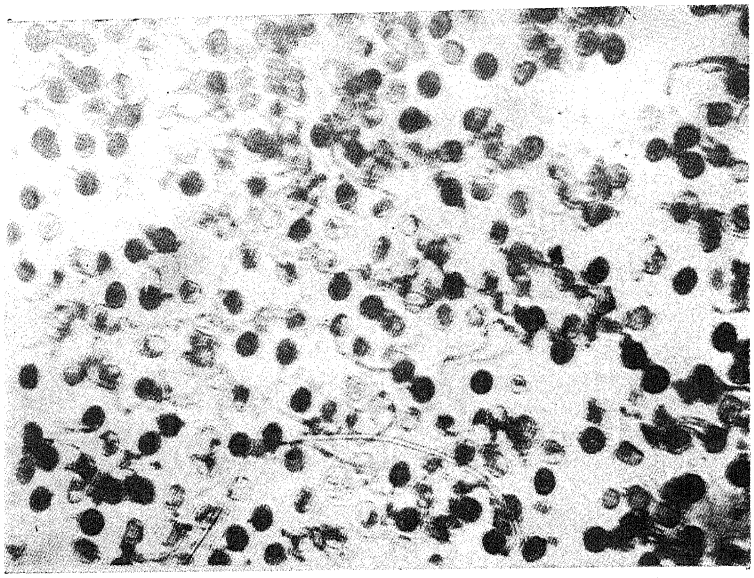
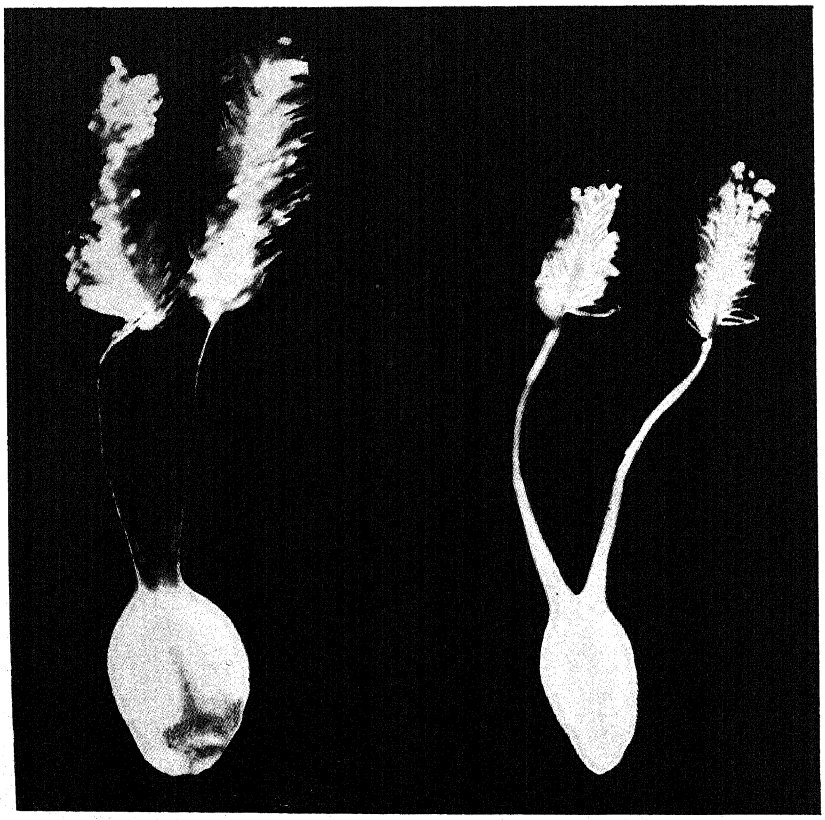


FIG. 1.— Germinating pollen of *S. margaritiferum* (×100)



S. durra. *S. nervosum.*

FIG. 2.—Stigmas (×15).

Portions of stigmas added to the germinating culture medium did not show any preferential germination of pollen near their proximity and the pollen tubes betrayed no tendencies to grow towards them. The germination of pollen usually commences a quarter to half an hour after the pollen has been sown on the medium (Plate LXVIII). It takes three-four hours to complete the growth. A single pollen-tube is the rule. Branched tubes are met with rarely. It is a common occurrence for pollen-tubes to burst at various stages of their growth.

The response of different varieties to artificial germination was tried. The results are recorded in Table V. In this and subsequent tables, unless otherwise mentioned the material used is *S. durra* and the standard culture medium for germination was forty-one per cent sucrose with agar.

TABLE V

Germination of pollen of sorghum varieties in artificial media

Varieties	Number of pollen grains on the medium		Percentage of germination
	Sown	Germinated	
Summer (irrigated)—			
S. durra, var.			
Chinna Manjal	3,050	1,719	56·5
Sen Cholam	1,051	546	51·1
Cold weather—			
Night flowering.			
S. cernuum, var.			
Tella Jonna	8,760	5,580	57·0
S. durra, var.			
Peria Manjal	5,489	2,713	49·4
S. roxb. var. *hians.*			
Talai-virichan	8,840	3,833	43·3
S. nervosum, var.			
Irungu	5,557	1,902	34·2
Andropogon Sorghum, var. *technicus.*			
Broomcorn	2,977	447	15·0
Day flowering.			
S. margaritiferum, var.			
Zomba	10,132	9,040	88·8
Wild allies.			
Andropogon annulatus	6,104	4,074	66·7
Andropogon pertusus	4,410	449	10·2

The different varieties are thus seen to be extremely variable in their response to artificial germination of their pollen grains. The high germinations that were obtained are noteworthy in view of the usual poverty of response common in cereal pollen.

It was next attempted to compare the germination of pollen from bisexual and male flowers. It will be seen from Table VI that in the percentage of germination, the average length of the pollen-tube as well as the maximum length attained by the tubes, the pollen from bisexual spikelets proved to respond better than the pollen from the staminate flowers. Pollen gathered in warm weather showed retarded pollen-tube growth.

TABLE VI

Pollen from bisexual and male flowers

	Number of pollen grains on the medium		Percentage of germination	Average length of pollen-tube	Maximum length of pollen-tube
	Sown	Germinated			
				μ	μ
Pollen from—					
Bisexual flowers.	17,379	10,503	60·4	260	1,296
Male flowers .	17,142	6,464	37·7	92	642

The germination percentage as well as the germination energy of pollen from staminate flowers is seen to be less than that of the hermaphrodite flowers. Seed-setting with these two kinds of pollen was tried on a small scale, with the following results.

TABLE VII

Pollen from bisexual and male flowers—seed-setting

	Number of flowers emasculated and pollinated	Number of seeds set	Percentage of seed-setting
Pollen from bisexual flowers . . .	73	35	48·0
Pollen from male flowers	79	14	17·7

Germinations were tried of pollen from anthers taken from the different parts of the ear-head. Those from the base of the ear-head showed the longest pollen-tube growth.

Pollen being available from 2 A. M., it was next sought to find out the maximum germination energy of pollen grains gathered and set for germination at hourly intervals from 2 A. M. to 10 A. M.

TABLE VIII

Influence of the time of day on pollen germination

Time at which cultures were set up	Number of pollen grains on the medium		Percentage of pollen germinated	Average length of pollen-tube	Maximum length of pollen-tube
	Sown	Germinated			
				μ	μ
2 A. M. . .	1,735	541	31·2	33	74
3 ,, . .	2,042	830	40·6	59	197
4 ,, . .	1,696	630	37·1	60	383
5 ,, . .	2,228	1,060	47·6	79	469
6 ,, . .	2,526	1,438	56·9	96	617
7 ,, .	1,947	1,012	51·4	165	827
8 ,, .	2,135	1,192	55·9	130	1,271
9 ,, . .	1,831	614	35·1	123	469
10 ,, . .	1,566	333	21·2	123	864

A steady increase is noticed till 8 A. M., when the maximum is reached, after which there is a decline. So also in the rate of growth of the pollen-tube.

Similar germinations were made with pollen from staminate flowers with no appreciable differences except the absence of the drop at 9 A. M. and 10 A. M.

Pollen from mature unopened flowers gave a normal germination response.

Temperature.—To determine the effect of temperature on pollen growth pollen was sown on the culture medium and grown at varying controlled artificial temperatures The germination records were taken after eight hours.

TABLE IX

Effect of temperature on the viability of pollen

(Control—27°C.)

Temperatures	Number of pollen grains on the medium		Percentage of germination
	Sown	Germinated	
10°C.	3,161	2,628	83·1
30°C.	1,680	954	56·6
35°C.	2,522	1,271	50·4
40°C.	2,699	1,285	47·6
50°C.	1,236	163	13·1

Low temperatures have a distinctly beneficial effect on pollen growth. As much as twenty-five per cent over those of the control temperature could be claimed in their favour. This accounts for cold days and the colder hours of the day tending to be more favourable for pollen germination. The figures show that high temperatures up to 35°C. ensure about fifty per cent germination, that at 40°C. this percentage is likely to be kept up and that an increase to 50°C. gives a sudden drop in germination.

Light.—Fresh pollen was carefully folded in two paper packets and kept in a beaker in moist chambers. A black paper wrapped well round one of the moist chambers cut off all light. Samples were sown from the control as well as that in darkness after forty-eight hours.

TABLE X

Effect of light on pollen

	Number of pollen grains on the medium		Percentage of germination
	Sown	Germinated	
Light	1,296	367	28·3
,, (duplicate)	1,492	392	27·4
,, (triplicate)	1,454	395	27·2
Darkness	1,328	44	3·3
,, (duplicate)	1,550	47	3·0
,, (triplicate)	1,242	*Nil*	*Nil*

The inhibiting effects of darkness on pollen germination are patent. The effect of light on pollen growth in artificial media being thus determined, its effect on growth on stigma was next attempted. It was found that flowers pollinated with pollen exposed to light resulted in thirty-six per cent of successful seed-setting while those dusted with pollen from darkness for twenty-four hours resulted in complete sterility. This favourable effect of light on pollen germination has to be remembered in connection with the higher germination percentage of the day flowering *S. margaritiferum*.

Growth of the pollen-tube.—A great difficulty in following up the progress of the growth of the pollen-tube is its tendency to burst. This may happen at any stage of the observation and is not surprising in view of the delicate equipoise that is necessary between the culture medium and the contents of the pollen-tube. A slight disturbance in the balance is enough to arrest growth and the tubes burst. This increases the difficulties of observation and many tubes had to be abandoned on this account.

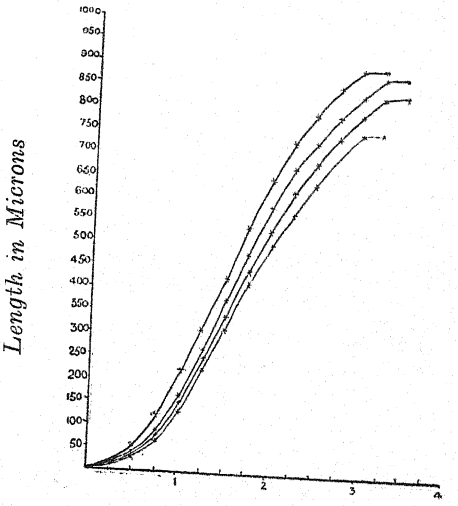


Fig. 1. Growth curves of pollen-tubes from bisexual flowers—A. S. 3065.

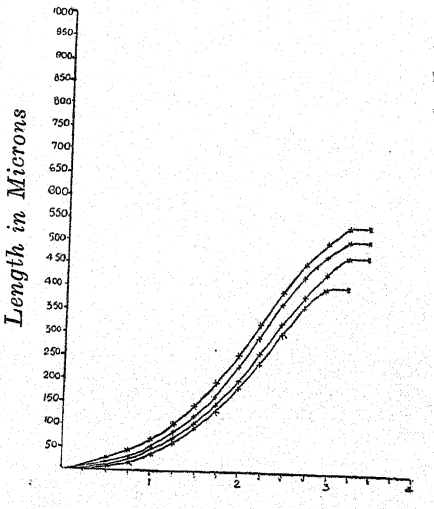


Fig 2. Growth curves of pollen-tubes from staminate flowers—A. S. 3065.

The growth curve of four pollen-tubes from bisexual flowers have been plotted in the graphs (Figs. 1 and 2). They all show the same general tendency—a preliminary period of growth, a rapid elongation and a gradual decline—giving the curve a somewhat shallow S shape. The first hour of growth is not very rapid but after that it gathers momentum and the greatest elongation is about that time. The decline is more gradual than the earlier acceleration.

The growth curve of the pollen from the staminate flowers has the same general shape and tendency but the rate of growth is different. It is not only slow at first, but continues at a slow pace for a much longer period. After this belated period of initial growth some speed is attained and as in the other cases, is followed by a gradual decline. The tubes are never able to reach the same length as those recorded for the pollen from bisexual flowers.

In nature, therefore, the pollen from staminate flowers is considerably handicapped. It is not numerous, is comparatively poor in germination, and slow and feeble in growth, rarely attains the length of the tubes of the pollen from bisexual flowers, and is consequently not so effective in fertilisation. These handicaps are in tune with the general degeneracy of the pedicelled spikelet.

Nucleus.—In most cases of germinating pollen, the lengths of pollen-tubes obtained in artificial cultures are negligible compared with the distance these tubes have to traverse to reach the ovary. In sorghum also it was not possible to secure the requisite lengths. The maximum length of tube obtained was 1,300 μ as against the 5 mm. necessary to reach the ovary.

Due to the dense contents of the pollen grains of sorghum, in many cases, the nuclei did not take the stain. Belling's Iron-aceto-carmine I was used to stain the nuclei. Prior to the shedding of pollen, there is only one big nucleus in the pollen grain. Shed pollen shows a tube nucleus and two generative nuclei. It is, therefore, probable that this differentiation of nuclei takes place at the time of shedding. The tube nucleus is generally larger than the generative nuclei. Sometimes it does not take the stain and when it does stain, it is usually lighter than the generative nuclei. The tube nucleus is usually at the grain end. In a few cases in *Andropogon annulatus*, the tube nucleus migrates far into the tube. When this occurs all the three nuclei are found together. In *S. margaritiferum* and *Andropogon annulatus* the nuclei are more or less spindle-shaped. In *S. durra*, the nuclei are larger and more elongated. The position of the generative nuclei follows no general rule. They are generally separated but sometimes are found close together. They may be found anywhere along the entire length of the tube. It is not necessary for pollen-tubes to be particularly long for the nuclei to migrate. Even in short tubes they are in evidence. In many cases,

though a considerable length of pollen-tube is attained, the nuclei had not migrated. In ungerminated pollen grains, in artificial cultures, what appear to be normal nuclei for all outward appearances, are found. In the light of these observations it is difficult to assess the influence of the nuclei on pollen-tube growth.

Pollen longevity.—The problem of preserving the pollen for some time without adversely affecting its vitality is a very important one. In practical plant-breeding, it frequently happens that two different strains, which it is desired to cross, do not have a common flowering period. If, therefore, the pollen of one could be kept viable until the required time, a great practical difficulty would be solved. Apart from it, the problem compels interest.

According to Patel and Patel [1929], sorghum pollen is capable of germination for at least thirty hours. The method of storage is not given by them. Stephens and Quinby [1934] find at Chillicothe, United States of America, that after five hours sorghum pollen loses its viability.

The moisture-content of pollen of *S. durra* was estimated to be about fifty per cent by drying the pollen *in vacuo* over sulphuric acid at laboratory temperature and pressure until a constant weight was reached. Sorghum pollen exposed to the atmosphere shrivels in about half an hour. Pollination with shrunken pollen does not set seed. *In vitro* it swells but does not germinate. Desiccating agents like sulphuric acid and calcium chloride quickly kill the pollen. Pollen preserved in an atmosphere of carbon dioxide failed to germinate. Similarly under reduced pressure sorghum pollen failed to germinate though several successes in the case of other kinds of pollen have been reported.

In sorghum, saturated moist conditions were found not to be lethal. The pollen does not shrink. Small packets of pollen were kept in beakers and the whole enclosed in moist chambers. Under these conditions germinations (about 28 per cent) were obtained after forty-eight hours. But after that fungus attack killed the pollen. It is difficult to say whether moist conditions *per se* can preserve the pollen. But, however carefully the experiments were conducted, infection by fungi occurred ; the probabilities are that moisture is not a very efficient preservative agent, for the germination percentage is greatly reduced.

The effect of low temperatures was next tried. Small paper packets of pollen kept in open tubes were preserved in an ice chest at 10°C. This gave the best results and life lingered till even the sixth day. This is the longest period that could be got. All other attempts to further prolong life proved futile.

TABLE XI

Pollen longevity—in vitro

Pollen from bisexual flowers

Age of pollen	Number of pollen grains on the medium		Percentage of germination
	Sown	Germinated	
24 hours old	2,026	521	25·7
48 „ „	2,189	496	22·5
72 „ „	2,241	412	18·4
96 „ „	2,318	309	13·3
120 „ „	2,296	175	8·0
144 „ „	2,176	24	1·1

It was thought that possibly there might be better germination on the stigma, because pollen six days old may not react normally towards the medium prepared with reference to fresh pollen.

TABLE XII

Pollen longevity—on the stigma

Pollen from bisexual flowers

Age of pollen	Number of pollen grains on the medium		Percentage of germination
	Sown	Germinated	
24 hours old	1,114	204	18·3
48 „ „	1,520	180	11·8
72 „ „	1,087	58	5·4
96 „ „	832	9	1·1
120 „ „	830	2	0·2
144 „ „	1,447	2	0·1

For all ordinary purposes the germination capacity may be taken as an index of its general viability. But viability does not necessarily imply ability to fertilise. Further tests were, therefore, made.

TABLE XIII

Pollen longevity—seed-setting

Pollen stored at 10°C. in an ice-chamber

Pollen from bisexual flowers

Age of pollen	Number of flowers emasculated and pollinated	Number of seeds set	Percentage of seed-setting
24 hours old	38	10	26·3
72 „ „	42	13	21·0
96 „ „	46	2	4·3

The pollen is capable of fertilising up to the third day though in a very weak measure. Dead pollen differs little from living pollen in appearance. Germination test is the only sure guide as death does not alter the shape of the grains. Owing to the weak fertilization an abundance of pollen grains seems indicated as a necessity in the case of pollination deferred so long. This experience is in marked contrast to that of Stephens and Quinby [1934].

THE STIGMA

The stigma has not commanded the same attention as the pollen. The literature on it is very scanty. The stigma is a much more difficult material to make observations on and does not lend itself to the same easy handling as the pollen. Most of the work has, therefore, been on the main issue of stigmatic receptivity. Pollen detaches from the flower and is easily handled. The stigma has the disability of being stationary. This difficulty was minimised by cutting off the ear-head and placing the stalk in water. It was noticed that this did not interfere with the opening of the flower and attendant phenomena for a considerable time. By this method, it was possible to easily study the reactions of the stigma under different experimental conditions.

Variations in the style and stigma.—The details of the floral morphology of sorghum have already been given [Rangaswami Ayyangar, and Panduranga Rao, 1931]. The stigma have the shape of a truncated spindle. Two shapes in this spindle can be made out—a brush like type which is the commonest and a bushy one characteristic of *S. roxb.* var. *hians* and *S. nervosum*. The middle branches in a stigma are the longest. The length of branches diminishes gradually towards both ends ; the topmost branch is, however, shorter than the lowest.

TABLE XIV

Length of stigmatic branches

(Average of 100 readings)

Varieties	Length of stigmatic branches at		
	Base	Middle	Top
	μ	μ	μ
S. durra	370	555	222
S. cernuum	344	530	185
S. nervosum	308	456	173
S. roxb. var. *hians*	246	456	197
S. margaritiferum	185	344	98
A. S. var. *technicus*	173	344	123

Variations have been found in the lengths of the style and stigmatic portions and in the combined length of the style and stigma. A typical pure line from each of the following varieties was taken and measurements were recorded (average of 100 readings).

TABLE XV

Variation in the length of style and stigma

Varieties	Length of		Total length of style and stigma mm.	Ratio of style and stigma
	Style mm.	Stigma mm.		
S. cernuum	2·25	2·25	4·50	1 : 1
S. durra	2·00	2·00	4 00	1 : 1
S. margaritiferum	1·75	1·75	3·50	1 : 1
A. S. var. *technicus*	1·50	1·50	3·00	1 : 1
S. roxb. var. *hians.*	2·50	1·25	3·75	2 : 1
S. nervosum	2·50	1·00	3·50	2·5 : 1

It will be seen that the combined lengths of the style and stigma is the greatest in *S. cernuum* and *S. durra* ; most of the cultivated varieties belong to the latter. This length is least in *S. nervosum*, the reed-like fodder variety in which the grains just peep out of the glumes. The other varieties have intermediate lengths. In *S. durra*, *S. cernuum*, *S. margaritiferum*, and *A. S.* var. *technicus*, the style and stigma are equal in length. In *S. roxb.* var. *hians*, and *S. nervosum*, the length

of the stigma is short and is about a third of the combined length of the style and stigma (Plate LXVIII, Fig. 2). An interesting correlation between the length of the subule and column of the awn and of the style and stigmatic zones of the stigma has been recorded. [Rangaswamy Ayyangar and Panduranga Rao, 1935].

Receptivity.—The receptivity of the stigma and the growth of the pollen on and through it was studied. Stigmas were chosen from flowers that open next morning. From the panicle all but twenty or thirty flowers were clipped off. The remaining flowers were emasculated in the evening and pollinated the next morning. After eight hours the germinating pollen was studied after staining. The stain used was 0·08 grm. of cotton blue in 100 c.c. of lactic phenol diluted with an equal quantity of water. The pollen-tubes and grains took the stain in about half an hour. The pollen may germinate on the stigma, but seed-setting need not necessarily follow. In these studies, wherever possible, seed-setting was studied concurrently with pollen germination. So far as stigma receptivity in sorghum is concerned, pollen germination leads to fertilisation.

Temperature.—After emasculation one panicle was kept in the incubator at 10°, 30° and 34°C. Another panicle was kept outside as control. The next morning fresh pollen was dusted on both and eight hours later the stigmas were killed. Counts of pollen germinated and ungerminated on the stigma were made with the aid of an Okular Netz micrometer (Zeiss).

TABLE XVI

Effect of temperature on the receptivity of the stigma

Temperature	No. of pollen grains on the stigma		Percentage of germination
	Sown	Germinated	
10°C.	2,885	2,690	93·2
Control (26·5°C.)	3,177	2,661	83·7
30°C.	1,426	846	59·0
Control (26·5°C.)	2,443	2,109	86·0
34°C.	4,231	1,004	23·7
Control (26·5°C.)	7,857	7,403	94·0

The results show the adverse effect of a temperature of 30°C. and over on the stigma and the favourableness of the lower temperature. Low temperature is thus found to be favourable both for the germination of pollen and the receptivity of the stigma.

Humidity.—Fresh pollen was dusted on the stigmas kept in a moist chamber under saturated moist conditions overnight. Counts show that such humid conditions reduce the receptivity of the stigma.

TABLE XVII

Effect of humidity on the receptivity of stigma

Treatment	Number of pollen grains on the stigma		Percentage of germination
	Sown	Germinated	
Saturated moisture	21,877	4,090	18·7
Control	7,857	7,403	94·0

Desiccation.—For the moist chamber in the above experiment a desiccator with calcium chloride was substituted. This desiccation of the atmosphere for about twenty hours had no effect on its receptivity to fresh pollen applied after such desiccation.

TABLE XVIII

Effect of desiccation on the receptivity of stigma

Treatment	Number of fresh pollen grains on the stigma		Percentage of germination
	Sown	Germinated	
Dry	2,784	2,462	88·4
„ (duplicate)	3,096	2,756	89·0
„ (triplicate)	2,761	2,521	91·0
Control	2,985	2,765	92·6
„ (duplicate)	3,111	2,851	91·6
„ (triplicate)	3,165	2,850	90·0

Light.—To test the effect of light on the stigma on the plant in the field two emasculated sets of flowers on the same panicle were chosen. One of these was wrapped in a fine tissue-paper bag and the other in a fine black paper bag, so as to cut off all light. Fifteen hours later, they were pollinated. The germination figures show that darkness does not favour the receptivity of the stigma. Seed-setting trials confirmed the above experience.

TABLE XIX

(*a*) *Effect of light on the receptivity of stigma*

(In darkness for 15 hours prior to pollination)

Treatment	Number of pollen grains on the stigma		Percentage of germination
	Sown	Germinated	
Light	2,024	1,880	92·8
„ (duplicate)	2,146	1,941	90·4
Darkness	1,964	857	43·4
„ (duplicate)	2,007	989	49·2

(*b*) *Effect of light on stigma and the resultant seed-setting*

(In darkness for 24 hours prior to pollination)

Treatment	Number of flowers emasculated and pollinated	Number of seeds set	Percentage of seed settin
Light	36	22	61·1
Darkness	33	5	15·1

Though the largest anthesis is as early as 2 A. M., when the mass of liberated pollen escapes the devastating influences of desiccation and gets an assured lodging in the stigmatic feathers, the optimum germination of such pollen gets deferred to daybreak and the availability of light. This definite differential behaviour in the receptivity of the stigma passed through darkness raises the interesting question of the delicacy of response to light even of so weak an intensity as that of the moon's.

Pollen-prepotency.—The differences in the size and colour of pollen in sorghum are not marked enough to effect a pollination with a combination of different kinds of pollen and test pollen-prepotency on the same stigma. It was, therefore necessary to emasculate and dust two different sets of flowers on the same panicle. One set was dusted with its own pollen and the other with foreign pollen.

TABLE XX

(*a*) *Pollen-prepotency—Pollen germination*

	Number of pollen grains on the stigma		Percentage of germination
	Sown	Germinated	
Own pollen (from yellow grained variety) .	1,834	1,259	68·1
Own pollen (from yellow grained variety) (duplicate).	2,018	1,486	73·6
Foreign pollen (from red grained variety) .	2,006	1,846	93·0
Foreign pollen (from red grained variety) (duplicate).	2,214	2,076	93·3
Foreign pollen (from white grained variety) .	1,905	1,737	90·6
Foreign pollen (from white grained variety) (duplicate).	2,322	2,194	94·4

(*b*) *Pollen-prepotency—Seed-setting*

	Number of flowers emasculated and pollinated	Number of seeds set	Percentage of seed-setting
Own pollen (from yellow grained variety).	35	17	48·6
Foreign pollen (from red grained variety).	30	20	66·6
Foreign pollen (from white grained variety).	43	27	62·8

It will be seen that foreign pollen is favoured.

Varietal differences in receptivity.—Stigma-receptivity in various varieties was tried both for pollination and seed-setting. Two sets of ten plants from a variety were chosen. Pollen was dusted (after emasculation) to flowers on one plant every day. In one set the stigmas were killed eight hours after dusting and the other was allowed to set seed.

TABLE XXI

(a) Receptivity of stigma in varieties—pollen germination

Variety	Number of pollen grains on the stigma		Percentage of germination
	Sown	Germinated	
S. durra (var. *Tella Jonna*)—			
Stigmas 24 hours old	3,464	2,970	85·7
,, 48 ,, ,,	3,349	2,690	70·3
,, 72 ,, ,,	6,088	1,072	17·6
,, 96 ,, ,,	5,298	1,541	29·1
,, 120 ,, ,,	1,784	633	35·4
,, 144 ,, ,,	2,171	703	32·3
S. durra (var. *Peria Manjal*)—			
Stigmas 24 hours old	11,426	10,322	90·3
,, 48 ,, ,,	11,526	10,962	95·1
,, 72 ,, ,,	1,477	742	50·2
,, 96 ,, ,,	1,724	938	54·4
,, 120 ,, ,,	1,656	*Nil*	*Nil*
S. roxb. var. *hians* (var. *Talaivirichan*)			
Stigmas 24 hours old	2,414	2,121	87·8
,, 48 ,, ,,	697	336	48·2
,, 72 ,, ,,	557	231	41·4
,, 96 ,, ,,	124	49	39·5
,, 120 ,, ,,	321	*Nil*	*Nil*

TABLE XXI—*contd.*

(b) Receptivity of stigma in varieties—seed-setting

Variety	Number of flowers emasculated and pollinated	Number of seeds set	Percentage of seed-setting
Sorghum roxb. var. *hians* (var. *Talaivirichan*—duration 150 days)—			
Stigmas 24 hours old	35	26	74·3
,, 48 ,, ,,	37	15	40·5
,, 72 ,, ,,	29	13	45·0
,, 96 ,, ,,	33	3	9·0
S. durra. (var. *Peria Manjal*—duration 145 days)—			
Stigmas 24 hours old	99	41	41·4
,, 48 ,, ,,	63	26	41·3
,, 72 ,, ,,	45	13	29·0
,, 96 ,, ,,	57	12	21·0
S. durra. (var. *Chitrai Vellai*—duration 115 days)—			
Stigmas 24 hours old	59	33	56·0
,, 48 ,, ,,	69	30	43·5
,, 72 ,, ,,	60	11	18·3
,, 96 ,, ,,	57	4	7·0
,, 120 ,, ,,	59	2	3·4
S. durra. (var. *Chinna Manjal*—duration 105 days)—			
Stigmas 24 hours old	84	53	63·1
,, 48 ,, ,,	82	52	63·4
,, 72 ,, ,,	42	11	26·2
,, 96 ,, ,,	99	7	7·4
,, 120 ,, ,,	177	14	7·9
S. durra. (var. *Vellai Cholam*—duration 95 days)—			
Stigmas 24 hours old	24	17	70·8
,, 48 ,, ,,	31	21	67·7
,, 72 ,, ,,	32	2	6·1
,, 96 ,, ,,	24	1	4·2

It will be noted that seed sets in stigmas that are forty-eight hours old after which there is only a weak setting leading to none on the sixth day. The experiences of Stephens and Quinby [1934] in which they record receptivity up to sixteen days is, therefore, a great divergence from this South Indian experience. It will be seen that the duration of a variety did not affect the duration of the receptivity of its stigma.

In a healthy stigma, the feathers are turgid and stand at right angles to the axis—which is a sign of their receptivity. Before the opening of the flower, they are not turgid and remain adpressed to the axis. Under adverse conditions—like disease, etc.,—the feathers are flaccid and have a withered look.

Conclusion.—The foregoing data show that darkness and humid conditions impair the viability of the pollen and the receptivity of the stigma. Low temperatures favour the receptivity of the stigma and the germination of pollen. Desiccation quickly kills the pollen but has no effect on stigma-receptivity. Sorghum is wind-pollinated and well adapted to arid rigions ; the stigma provides a large surface area to catch the pollen and suitable substratum for its germination (and protection from desiccation). The pollen and stigma are both delicate structures. The details of their delicate response to their environment show that a fine equipoise between the ability of the pollen to germinate and the stigma to be receptive has been achieved. A careful examination of the sorghum stigma showed no fluid excretions nor does it exert any tropistic influence. The role of the stigma, therefore, seems to be to maintain optimum moisture conditions for pollen germination.

Summary

In sorghum the anthers vary in size but not in shape. The commonest cultivated varieties possess the biggest anthers. The pollen grains also vary in size and roughly parallel anther-size differences. "Dummy pollen" is peculiar to sorghum. It occurs in *Pennisetum typhoideum* also. Its incidence and functions are detailed. It is found that its incidence in numbers large enough to ensure anther opening, is a simple dominant to poor incidence leading to non-dehiscence and sterility.

Sorghum pollen is highly sensitive to the medium in which it is grown. Forty-one per cent sucrose with 0·75 (summer) and 1·5 (winter) per cent shred agar is found to be the best medium for the artificial germination of pollen. Germinations are better on cloudy, humid days than on hot, dry days. Considerable variation is found in the germination percentage of cultivated varieties. Compared with the night-flowering varieties, the day-flowering variety gave a much higher germination percentage. Pollen from staminate flowers has a distinctly lower percentage of germination and the tube length is comparatively shorter. Though pollen germinates from 2—10 A. M., best germinations are obtained from material between 7 A. M. and 8 A. M. Low temperatures have a distinctly beneficial effect on pollen germination. Up to 40°C. the germination is fairly good but beyond that there is a sudden drop in germination. Darkness inhibits the germination of pollen. The pollen grain has a tube nucleus and two generative nuclei. In *S. margaritiferum* and *Andropogon annulatus*, the nuclei are spindle-shaped. In *S. durra* they are larger and more elongated. In the initial stages, the pollen-tube from the pollen of staminate flowers grows slower than that in bisexual flowers. To attain its maximum length, the pollen-tube takes three to four hours in artificial

cultures. Under saturated conditions for forty-eight hours, twenty-eight per cent of the pollen remains viable. Pollen is capable of fertilising up to the third day though in a very weak measure (21 per cent).

Stigmas have the shape of truncated spindles. Two types of stigmas—a brush-like common one and a bushy one characteristic of *S. roxb.* var. *hians.* and *S. nervosum*—are met with. The germination of pollen on stigmas is very high. Stigma-receptivity is impaired by rise of temperature or humidity and darkness inhibits it though desiccation of the atmosphere does not seem to affect it. Seeds set in stigmas that are forty-eight hours old after which there is only a weak setting leading to none on the sixth day. The percentage of setting decreases with the age of the stigma. The duration of a variety does not seem to influence the duration of the receptivity of its stigma. The role of the stigma seems to be primarily to maintain optimum moisture-conditions for pollen germination.

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BLIGHT AND HOLLOW-STEM OF SORGHUM

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(With Plates LXIX-LXXII and one text-figure)

Rhizoctonia bataticola (Taub.) Butler, or its pycnidial stage *Macrophomina phaseoli* (Maubl.) Ashby, has a very wide host range, yet so far this fungus has not been reported to cause disease in *jowar, Andropogon sorghum.* In 1930 [Uppal, 1931] *jowar* plants in many fields in the neighbourhood of Broach in Gujarat were observed to show signs of early maturity, and on closer examination were found to have become stiff and dried up. When stalks of such plants were split open, the pith and fibres were found studded with numerous black sclerotial bodies. In the following year a seedling blight of *jowar* appeared in an epidemic form in East Deccan following periods of heavy late rains and high temperatures, and the diseased plants also exhibited the general symptoms of drying and premature ripening. It seems very probable, however, that the disease has existed in the Bombay Presidency for many years, although it has not so far been reported elsewhere in this country. Recently Mackie [1932] has described a similar disease in maize from California.

THE DISEASE

The disease is common in *rabi jowar* on the *bhata* soils along the Nerbudda river in Broach and near Mohol in East Deccan. The *bhata* soils are sandy loam to silt loam and are formed every year by the deposition of silt and other fine material brought with flood waters during the monsoon. They are deficient in humus and have low power of retaining moisture. The soils in East Deccan, however, are medium black and are retentive of moisture.

Infection in *kharif jowar* has never been observed as evidently the environmental conditions during the monsoon are not favourable. The seriousness of the disease in *rabi jowar,* however, is greatly modified by the character of the

season in any particular year, infection becoming general when heavy rains and high temperatures are experienced in October or early November. In such years seedling blight is very common in East Deccan, resulting in irregularity of stand and subsequent reduction in yield. Observations during the past five years have shown that the disease is, on the whole, of sufficient economic importance.

Symptoms.—Generally there is no external evidence of the disease until the plant approaches maturity. At this time infected plants show premature ripening, but the ear-heads are poorly developed (Pl. LXIX, fig. A). The stalks are weak as they are hollow inside, and may break under the force of strong wind. These stalks are easily shaken by wind and, as they strike against each other, they produce a sound somewhat reminiscent of the beating together of hollow, dry sticks, and hence the disease is aptly called *khadkhadia* in Broach.

When the infected stalk is split open, numerous black bodies are seen clustered round the fibrovascular bundles and in the medulla or pith ; they are not present in the inner cortex. These small bodies are the sclerotia of the fungus and are found for considerable distances up in the stem and down in the roots (Pl. LXIX, fig. B1). The development of the sclerotial bodies in the pith causes this tissue to disintegrate, resulting in the longitudinal splitting of the internal tissue along the bundles into masses of fibres (Pl. LXIX, fig. B2). The development of sclerotia on the fibrovascular bundles interferes with the normal movements of water and food materials in the plant, resulting in premature ripening and shrivelling of grains in poorly developed ears (Pl. LXIX, fig. C). The disintegration of medulla or pith, on the other hand, weakens the plant which may break under the force of wind.

The fungus enters the plant through small feeding roots and ascends the stem for considerable distances and in some cases may be traced up to the ear-head. In the earliest stage of the disease the cortical tissue at the collar has a flaccid appearance, later turns reddish brown and ultimately rots away. If environmental conditions may turn out very favourable in the early stage of attack, seedling blight becomes very destructive, and the cultivator attributes depletion in stand to poor germination or to hot weather injury.

Inoculation experiments

Numerous isolations have been made from diseased *jowar* plants collected in widely separated localities, and in all cases a fungus has been obtained that on potato-dextrose agar produces mycelium similar to that of *Rhizoctonia*, and later forms black sclerotia and in some strains also produces pycnidia containing pycnospores. This fungus is indistinguishable from *Macrophomina phaseoli* (Maubl.) Ashby, the pycnidial stage of *Rhizoctonia bataticola* (Taub.) Butler, which is commonly isolated from cotton plants suffering from the so-called root-rot.

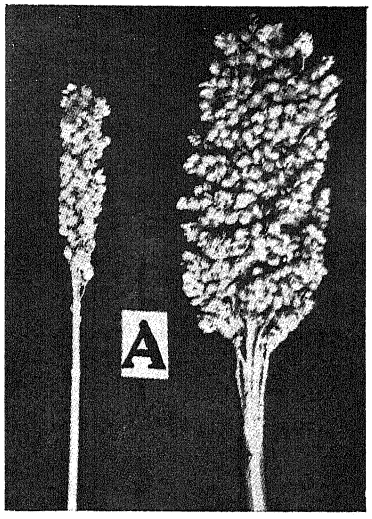


FIG. A. *Left.* An ear-head from an infected plant. *Right.* A normal ear-head.

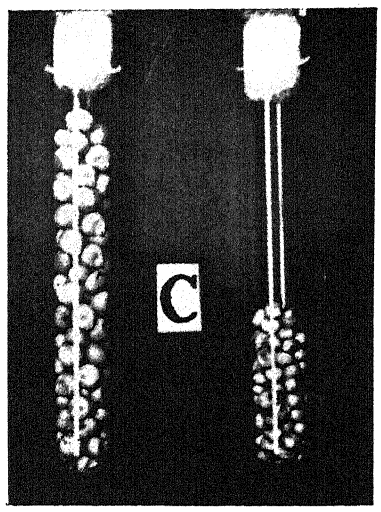


FIG. C. *Left* 100 seeds from a normal ear-head. *Right.* 100 shrivelled and poorly developed seeds from a diseased ear-head.

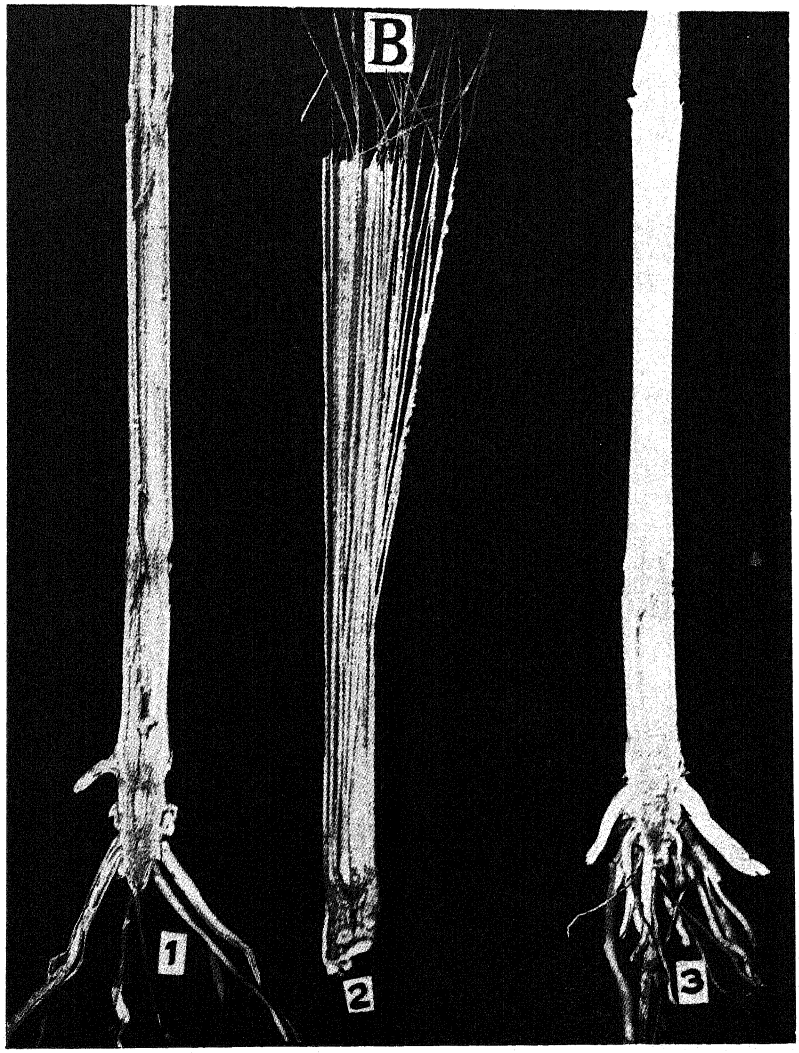


FIG. B. (1). A diseased plant showing sclerotial bodies in the pith and in the roots; (2) Disintegration of pith and separation of vascular bundles. Sclerotia are attached to bundles; (3) A healthy stalk split open.

FIG. 1. Healthy seedlings.

FIG. 2. Artificially infected seedlings showing browning of the stem at the collar.

In order to test its pathogenicity, the fungus (Broach strain, *i.e.*, the isolate of *Macrophomina phaseoli* from Broach) was multiplied on Richard's agar in giant Erlenmeyer flasks, and the resulting mycelial growth was uniformly mixed with sterilised river soil, which was then put into large glazed containers. *Jowar* was sown in these containers ; four weeks after sowing the seedling plants were examined, and six out of twenty plants showed root infection and browning of the stem at the collar (Pl. LXX, fig. 2) and gave a pure culture of the fungus on isolation. The control plants remained healthy.

In another test *jowar* plants were grown in Knop solution in Roux tubes, and small bits of agar culture of the fungus (Broach strain and Mohol strain, the latter being an isolate from Mohol) were placed on cotton wool close to the roots. The tubes were incubated at 30 to 35° C. In a few days the seedlings collapsed, the roots turned brown, and numerous small black bodies appeared in the stem and roots. Microscopic examination of these plants showed that, in addition to sclerotial bodies, pycnidia were also produced by Mohol strain. Control plants remained healthy. The results are recorded in Table I.

TABLE I

Macrophomina phaseoli *pathogenic on* jowar *plants grown in Roux tubes*

Fungus	No. plants inoculated	Per cent of infection	Remarks
Broach strain . .	6	100	Sclerotia produced
Mohol strain . . .	6	100	Sclerotia and pycnidia produced
Control	8	0	Plants healthy

Effect of soil-temperature on infection.—Field observations indicated that *Macrophomina* disease in *jowar* usually becomes serious during and after periods of high temperatures. Experiments were, therefore, made to determine the relation of soil-temperature to infection. The results of one of these experiments are described below.

Inoculum was prepared by growing the fungus (Broach strain) on Richard's agar, and a definite quantity of it was added to sterilised soil in each galvanised iron container. Temperatures ranging from 20 to 40° C. were maintained constant in Soil Temperature Tanks of Wisconsin type. *Jowar* plants were grown in the containers at different soil-temperatures for six weeks, after which period the containers were removed to a glasshouse and plants allowed to grow to maturity under ordinary conditions. After the plants had produced ear-heads, they were split open and the presence or absence of sclerotial bodies in the stem and roots was noted in each case. Control plants were grown at each temperature and were similarly treated except that no inoculum was added to the soil. The results are summarised in Table II.

TABLE II

Relation of soil-temperature to infection of jowar

Soil-temperature (°C.)	No. plants exposed to infection	Per cent of infection
20·0	34	0·0
30·0	35	0·0
33·0	28	7·1
35·5	19	36·8
37·5	23	13·0
40·0	25	0·0

It will be seen from Table II that 35·5° C. is the optimum temperature of infection, which falls off appreciably below and above this level. At 30° C. and below no disease developed in plants which made normal growth. There was also no infection at 40° C., but plants suffered from the effects of high temperature and made poor growth. Seedling blight occurred at 35·5° and 37·5° C., but this type of injury was not common at 33° C. In all cases control plants remained healthy and produced normal ear-heads.

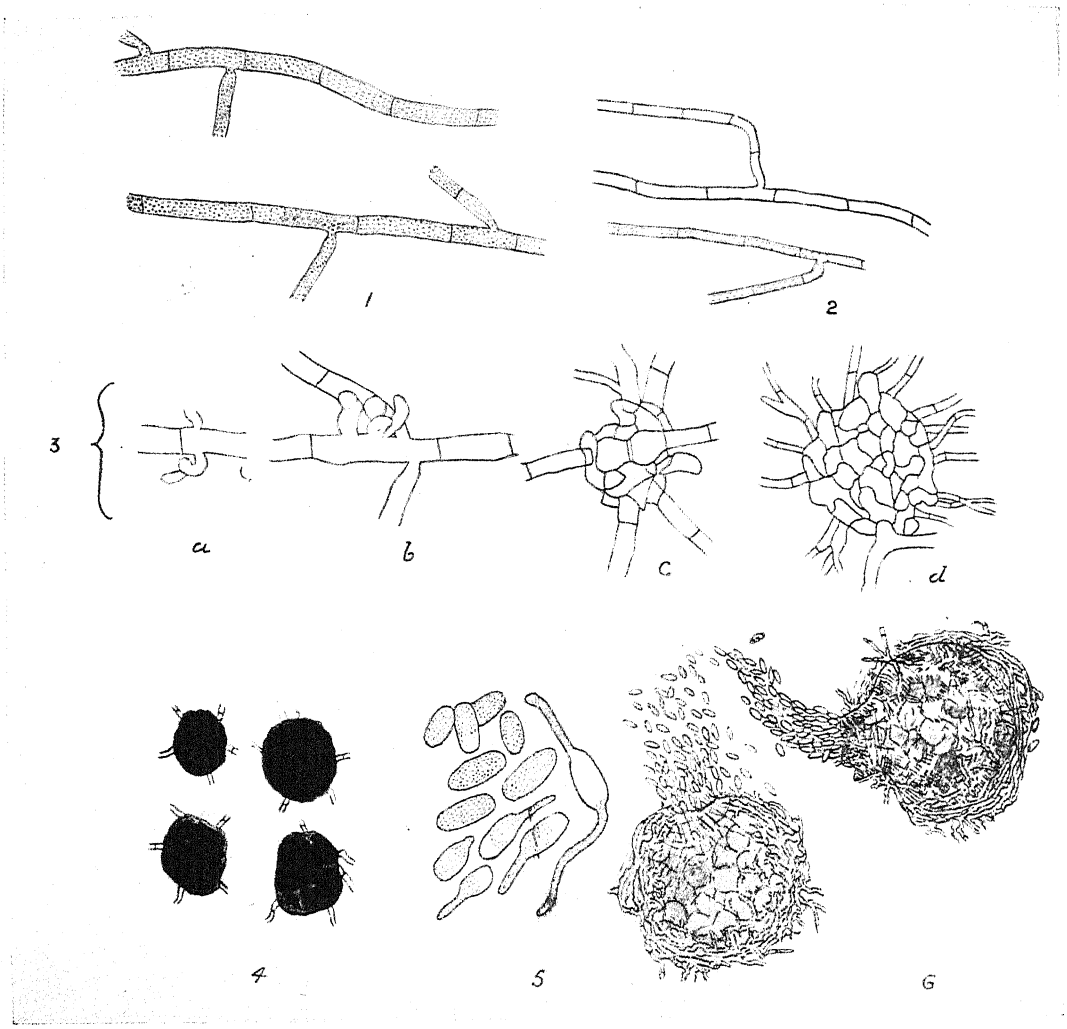
Effect of soil-moisture on infection.—Experiments were further made to determine the relation of soil-moisture to infection in correlation with optimum soil-temperature (35° C.). These experiments included a range of soil-moistures from twenty to sixty-five per cent based on the water-holding capacity of the soil. All plants were grown in different soil-moistures in Soil Temperature Tanks for six weeks and then grown to maturity under ordinary conditions. The results are recorded in Table III.

TABLE III

Relation of soil-moisture to infection of jowar

Water-holding capacity of soil	No. plants exposed to infection	Per cent of infection
20	11	0·0
30	11	0·0
40	11	0·0
50	9	0·0
65	9	33·3

It will be seen from Table III that high amounts of moisture in the soil are favourable to infection, the fungus becoming completely inactive in fifty per cent



FIGS. 1. Young hyphae of *Macrophomina phaseoli* colourless and full of protoplasm showing characteristic branching. (×560). 2. Old hyphae thin and with little or no protoplasm. (×560). 3. *a-d* Successive stages in the formation of sclerotia. (×560). 4. Black, carbonaceous sclerotia. (×150). 5. Showing range of size and shape of conidia. Also germinating conidia. (×560). 6. Pycnidia with a truncate ostiolum, and conidia. (×110).

water-holding capacity of the soil or lower moisture-content. Since soil-moisture is generally below the favourable level of infection under the normal range of field conditions during the *rabi* season in East Deccan, high temperatures alone do not seem to be sufficient to induce infection under conditions of low soil-moisture content. Soil-temperature and soil-moisture are thus the limiting factors, the fluctuations of which determine the occurrence and severity of infection of *jowar*. These results confirm the common observation that the disease is prevalent in *rabi jowar* following periods of heavy rains and high temperatures. In years when no late rains are received, the disease does not appear in East Deccan although soil-temperature in October and early November is generally favourable to infection.

It is also the general observation that clayey soils are more favourable to infection because of their ability to retain moisture, than loose sandy loams. In an experiment *jowar* plants were grown in clay and sandy loam at 35° C., and to these equal quantities of inoculum were added. The results in Table IV show that the percentage of infection in clayey soil is much higher than in sandy loam.

TABLE IV

Effect of type of soil on infection of jowar

Type of soil	No. of plants in test	Per cent of infection
Sandy loam . . .	20	10
Black soil (clay) . . .	20	85

MORPHOLOGICAL CHARACTERS OF THE FUNGUS

The morphological characters of *Macrophomina phaseoli* vary with the age of culture. When young, the hyphae are colourless and full of dense protoplasm ; they average about 8μ in diameter. Their branching is characteristic ; the branches lie almost parallel to the main hypha and are constricted at the point of union ; a wall is laid down a short distance from this point (Pl. LXXI, fig. 1). With age the hyphae become thin, brown and vacuolate, the lumen ultimately containing little or no protoplasm. The branches then lie almost at right angles to the main hypha (Pl. LXXI, fig. 2).

In young cultures the sclerotia become visible in forty-eight to seventy-two hours at about 27° C. They are formed from a hypha the cells of which repeatedly divide giving rise to masses of irregular and barrel-shaped cells. Secondary

branches arise and curl round one another so that a minute sclerotium is formed. These bodies rapidly grow and soon become black and carbonaceous (Pl. LXXI, fig. 4). A few stages in the formation of sclerotia are shown in the plate (Pl. LXXI, fig. 3 *a-d*).

Both Mohol and Broach strains form sclerotia freely on potato-dextrose agar and also show concentric zoning in plate culture (Pl. LXXII, fig. A). Saltations may arise spontaneously, and Plate LXXII shows a sterile form produced by Mohol strain, in which mycelium is submerged instead of aerial (Pl. LXXII, fig. B). The range of sclerotial size of Broach and Mohol strains is given in Table V.

TABLE V

Diameter of sclerotia of Macrophomina phaseoli

Fungus	Range	Mean
Broach strain . . .	40—220μ	130μ
Mohol strain	40—180μ	110μ

It will be seen from the table that Broach and Mohol strains have a mean sclerotial diameter of 130μ and 110μ respectively. Haigh [1930] has divided different strains of *Macrophomina phaseoli* into A, B and C groups on the basis of the mean sclerotial diameter in culture. According to this classification both Broach and Mohol strains fall in the C group having small sclerotia with a mean sclerotial diameter of about 120μ or less, although the upper limits of the range of sclerotial measurements, particularly of Broach strain, overlap the lower end of Haigh's B group having a mean sclerotial size of about 200μ.

Pycnidia are freely produced by Mohol strain on *jowar* seedlings grown in Roux tubes with Knop solution, but have never been observed in Broach strain. They are blackish brown with a truncate ostiolum and measure from about 150 to 290μ in diameter (Pl. LXXI, fig. 6). The pycnospores are mostly oval or elliptical in shape, hyaline, continuous and with a thin wall ; the content is finely granular (Pl. LXXI, fig. 5). In size they vary from 10 to 24μ × 6 to 10μ, mostly 14 to 20μ × 6 to 8μ ; these measurements agree with those given by Ashby [1927] for the fungus, although the conidia on *jowar* are somewhat smaller in length. In germination the conidia produce one or more germ-tubes (Pl. LXXI, fig. 5).

CULTURAL CHARACTERS OF THE FUNGUS

Growth at different temperatures.—The effect of temperature on the rate of growth of Broach and Mohol strains on Richard's agar was studied. Each strain

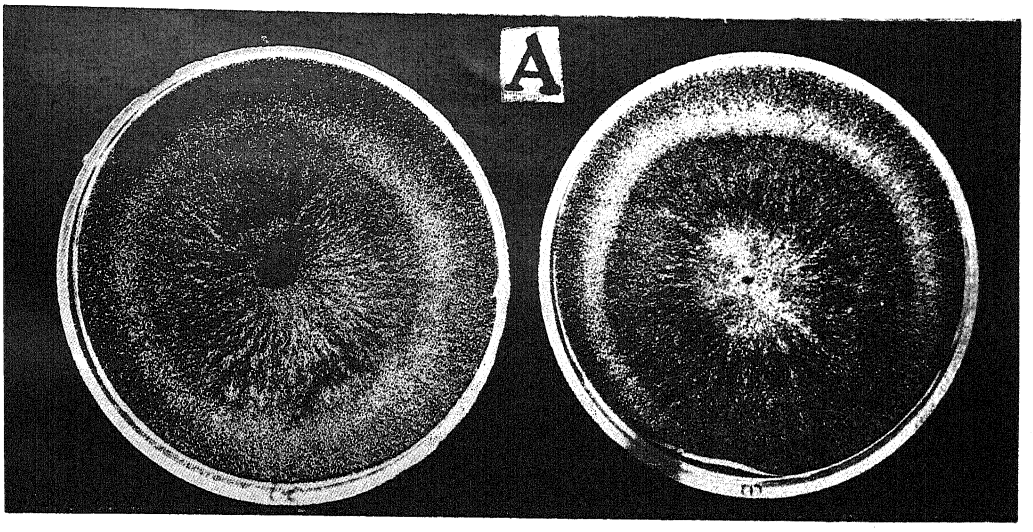


FIG. A. Cultures of Broach strain of *Macrophomina phaseoli* on potato-dextrose agar showing concentric zoning.

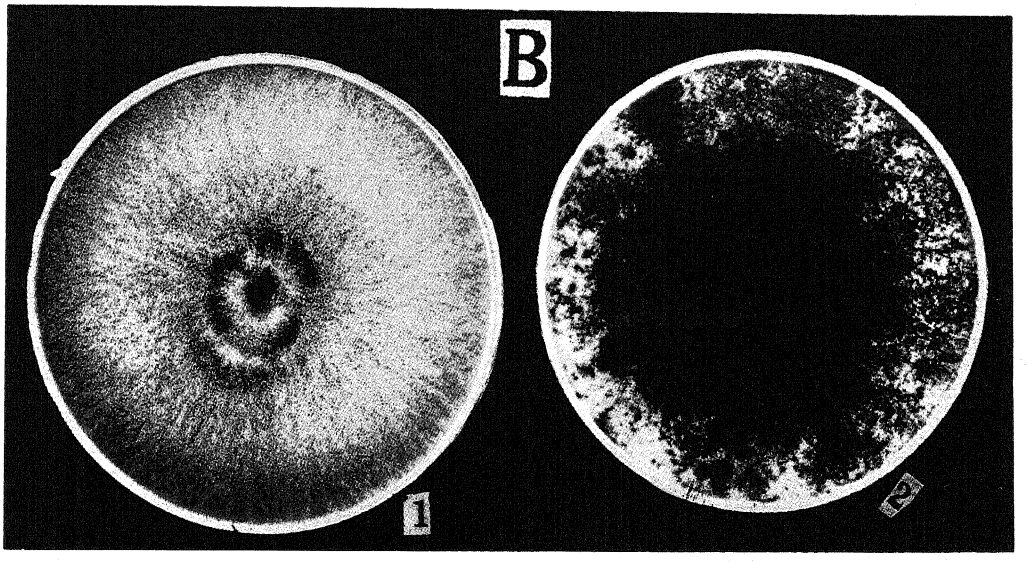
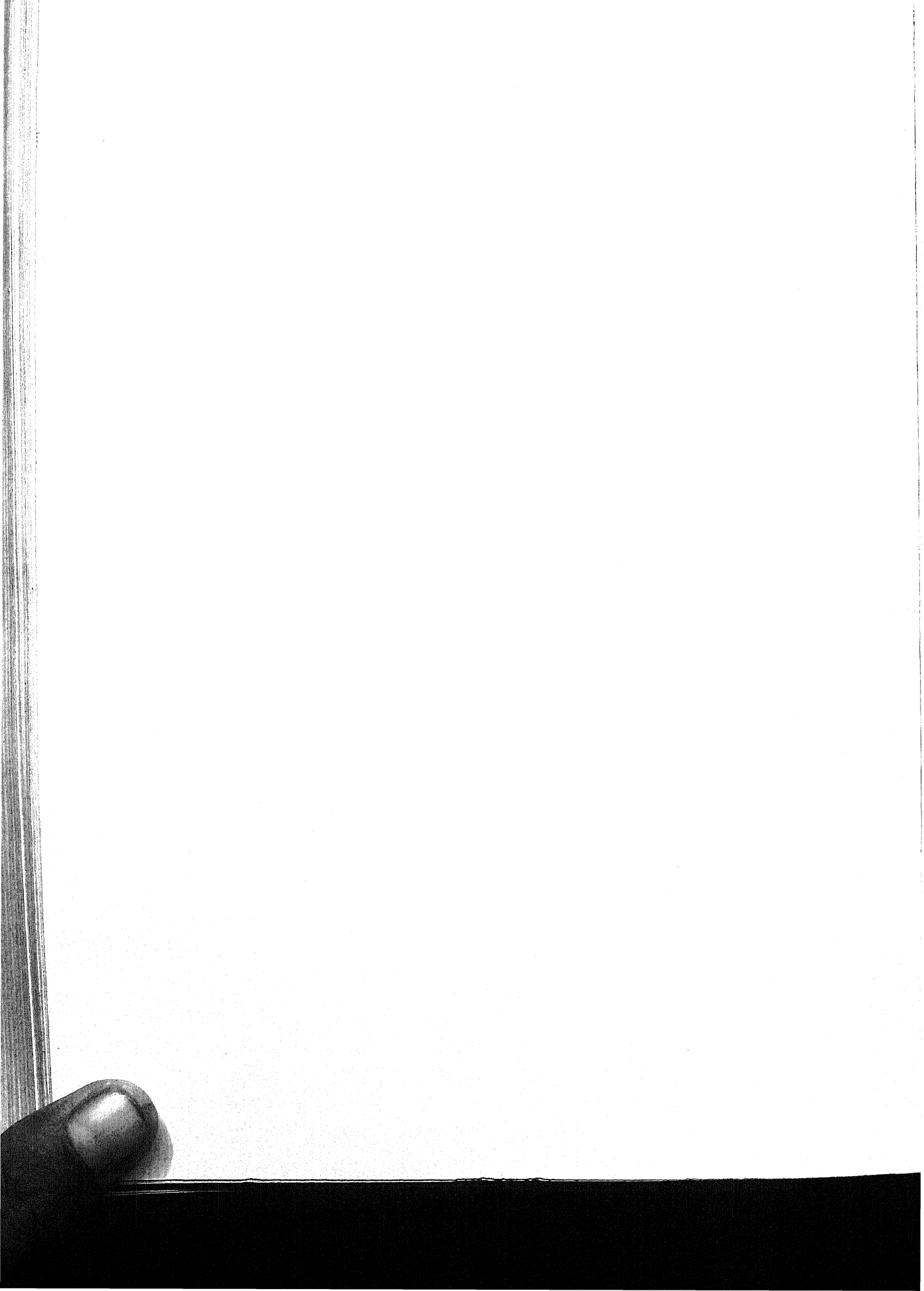


FIG. B. (1). Normal culture of Mohol strain showing aerial mycelium and sclerotia ; (2) Sterile saltant on potato-dextrose agar showing submerged dark strands of hyphae.



was grown in duplicate at different temperatures. Measurements of colony diameter were made daily for seven days ; but at optimum temperatures the fungus growth covered the whole plate at the end of three days. The curves showing graphically the rate of growth of the two strains at different temperatures at the end of three days are given in Fig. 1.

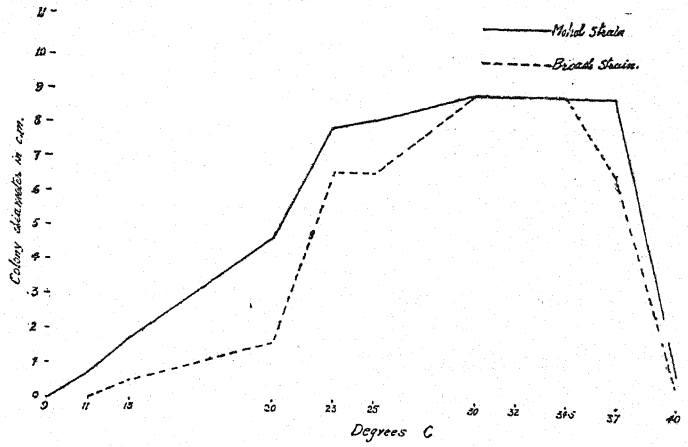


Fig. 1. Relation of temperature to radial growth of *Macrophomina phaseoli*.

In general, the two strains have about the same rate of growth at different temperatures, although Mohol strain grows faster than Broach strain. The optimum temperature of Broach strain lies between 30 and 34·5° C. and of Mohol strain between 30 and 37° C. ; at higher levels the radial growth of the two strains falls off sharply. The fungus also makes very slow growth at lower temperatures. Whilst Mohol and Broach strains made no growth at 9° and 11° C., respectively, at the end of two days, they had made slight growth after five days. At lower levels, however, growth was completely checked. At 40° the two strains make feeble growth in the beginning, but at the end of seven days colonies of Mohol strain measured 3·9 cm. in diameter, and those of Broach strain were hardly 1·9 cm.

Growth in liquid media.—A series of flasks was set up containing 500 c.c. quantities of each of the seven synthetic liquid media. The flasks were inoculated with Broach and Mohol strains and incubated at 30 to 32° C. ; duplicate tests were run in each case. At the end of fifteen days the cultures were filtered and the mycelial mats were washed and dried between blotting paper and then over calcium chloride *in vacuo* for a week. The mats were finally weighed (Table VI).

TABLE VI

Dry weight of mycelial mats of Broach and Mohol strains

Culture medium	Dry weight of mat in grms.		Remarks
	Broach strain	Mohol strain	
Meyer	3·430	4·140	Many sclerotia
Czapek	3·310	3·865	,,
Richard	2·900	3·380	,,
Pfeffer	2·257	3·140	,,
Fermi	0·510	1·410	Few sclerotia
Coons	0·640	0·840	,,
Cohn	0·290	0·620	Very few sclerotia

It will be seen that the two strains grow very well in Meyer's solution, forming an abundance of sclerotial bodies. Czapek's, Richard's and Pfeffer's solutions were also very suitable for growth and sclerotial formation. In Fermi's and Coons' solutions, however, the two strains make very poor growth, and the number of sclerotia formed is also very small. Cohn's solution seems to be the least suited both for growth and sclerotial formation. It appears that vigorous mycelial growth induces abundant sclerotial formation.

Broach strain does not grow as well as Mohol strain in different liquid media.

Growth on solid media.—Five solid media were tested. Equal quantities of each medium were poured deep in Petri dishes. All media were inoculated in duplicate with the two strains, and plates were incubated at 30-32° C. Measurements of colony diameter were made for seven days, but as the fungus growth covers the surface of plates in four days in some cases, figures of four days' growth only are recorded in Table VII.

TABLE VII

Growth of Broach and Mohol strains on solid culture media

Culture medium		Colony diameter in cm. after days			
		1st	2nd	3rd	4th
Quaker oats agar	M*	1·2	**4·2	7·2	9·0
	B	1·0	4·0	6·5	8·5
Potato-dextrose agar	M	0·5	2·2	**5·5	7·5
	B	1·0	2·5	4·5	6·0
Corn-meal agar	M	2·0	4·2	**6·2	9·0
	B	1·5	3·0	4·0	4·5
Richard's agar	M	0·0	0·2	**2·0	4·2
	B	0·2	1·2	2·5	5·0
Brown's agar	M	0·5	2·2	4·5	5·5
	B	0·5	1·7	3·7	4·5

* "M" stands for Mohol strain and "B" for Broach strain.
** First appearance of sclerotial bodies.

It will be seen from the table that, except on Richard's agar, Mohol strain grows faster than Broach strain. Quaker oats agar promotes good mycelial growth, and sclerotia appear in culture on the second day after inoculation. Sclerotia are also formed freely on potato-dextrose agar, corn-meal agar and Richard's agar, but do not appear until the third day after inoculation. On Brown's agar, however, no sclerotia were observed even until the ninth day after inoculation.

Growth in nutrient broth in relation to H-ion concentration.—Broach and Mohol strains were grown in nutrient broth adjusted to different reactions at 30-32° C. After fifteen days the dry weight of mycelial mats was determined and is shown in Table VIII.

TABLE VIII

Growth of Broach and Mohol strains in nutrient broth at different p*H*

*p*H	Dry weight of mat in mgrms.	
	Broach strain	Mohol strain
2·6	4	3
3·4	117	110
4·5	118	129
5·4	113	115
6·4	75	67
7·4	46	60
8·3	43	32
9·4	49	29
10·4	19	32
11·4	4	11
12·4	2	0

The two strains can tolerate a wide range of H-ion concentrations, whilst the range of optimum reaction appears to lie between *p*H 3·4 and 6·4, indicating that acid reactions favour growth of the fungus. Sclerotia are formed in culture at all reactions, but they are more numerous in acid than in alkaline reactions.

Growth on Dox's agar in relation to H-ion concentration.—Dox's agar was made up and adjusted to different *p*H values by the colorimetric method. Before pouring into plates, a suitable indicator-dye was added to each set of the medium. The two strains were then grown on the medium, and after incubation at 30-32°C., the plates were examined after every twenty-four hours for four days to note the colour changes in the medium below the fungus colonies. Thus, if a small amount of the fungus culture is transferred to a plate of Dox's agar of *p*H 12·4 and containing sterile cresol red, the colour of the medium below the colony turns slightly yellow after two days, indicating acid-production and a shift in reaction to about *p*H 8. Measurements of colony diameter are recorded in Table IX.

TABLE IX

Growth of Broach and Mohol strains on Dox's agar at different pH

*p*H		Colony diameter in cm. after days		
		1st	2nd	4th
2·6	M*	2·0	6·9	9·0
	B	1·6	4·2	8·5
3·4	M	2·7	7·2	9·0
	B	1·8	4·8	9·0
4·5	M	2·7	7·2	9·0
	B	2·0	5·0	9·0
5·4	M	2·8	7·1	9·0
	B	2·1	5·1	9·0
6·4	M	2·7	7·0	8·7
	B	2·0	4·5	8·7
7·6	M	2·1	6·8	8·9
	B	1·8	4·0	8·7
8·6	M	2·2	7·0	8·9
	B	1·7	4·0	8·6
9·6	M	2·3	6·6	8·9
	B	1·6	3·7	8·5
10·4	M	2·5	6·3	8·9
	B	1·6	3·2	8·2
11·4	M	2·3	5·9	8·5
	B	0·8	3·0	8·3
12·4	M	2·0	5·2	7·3
	B	0·6	2·9	6·4

* "M" stands for Mohol strain and "B" for Broach strain.

It will be seen that, as in the case of nutrient broth, the optimum reaction for the growth of the two strains (after 48 hours) appears to lie between *p*H 3·4 and 6·4. At the end of ninety-six hours, however, there was little or no difference in the rate of growth on Dox's agar at different reactions due to progressive change towards acidity of the more alkaline media. Sideris [1929] has shown that "acidic substances may be formed during metabolic activities at *p*H values above that of the isometabolic point of the substance concerned, and basic substances may be formed below this point". The rapid changes in *p*H value

induced in the agar substratum, however, may be due to lesser diffusion of the metabolic products of the organism in the solid than in the liquid medium, and this may explain the result that the growth on Dox's agar is practically normal after ninety-six hours at such higher *p*H values as *p*H 10·4 to 12·4. In acid reactions mycelial growth is aerial and fluffy, becoming more and more flat as the reaction shifts towards alkalinity. Sclerotia are not formed in highly alkaline or highly acid reactions, but are numerous between *p*H 5·4 and 6·4, their number decreasing as the medium becomes more acid or more alkaline. These results are in general agreement with those of West and Stuckey [1931] for *Macrophomina phaseoli* from cotton.

Relation of oxygen to vegetative growth.—An experiment was made to determine whether or not *Macrophomina phaseoli* can grow on culture media in the absence of free oxygen in the surrounding atmosphere. A small bit of a vigorously growing culture of the fungus was transferred to a plate of potato-dextrose agar, and the latter was then placed in a desiccator. For the elimination of oxygen from the desiccator, an alkaline solution of pyrogallol was used, which was prepared with one gram of pyrogallol and 10 c.c. of ten per cent potash-water for every 100 c. c. of air space [Smith, 1905].

The desiccators were incubated at room temperature for one month, but no growth was observed in the plates. However, when the plates were removed from the desiccators, distinct signs of growth were visible after two days. In another test the fungus was still viable after the plates had been in an oxygen-free atmosphere for two months. These results indicate that *Macrophomina phaseoli* can lie dormant in poorly aerated soils and may resume activity on the return of favourable conditions.

Summary

Macrophomina phaseoli is shown to be responsible for a seedling blight and hollow-stem of *jowar*, a disease which is new to the Bombay Presidency and has not so far been reported elsewhere. The disease is common on *bhata* soils in Broach and on medium black soils in East Deccan.

The fungus enters the plant through feeding roots and ascends the stem and may be traced up to the ear-head. There is no external evidence of the disease until the plant approaches maturity. At this time infected plants are found to have hollow stalks and produce a characteristic sound as they are forcibly shaken by wind; hence the disease has been given the name "hollow-stem". Seedling blight is also common and may become very destructive under conditions of high soil-temperature and high soil-moisture content.

The fungus normally forms sclerotia, but Mohol strain of the fungus also produces the pycnidial stage on *jowar* seedlings grown in Roux tubes.

The disease is most active at high soil-temperature (35°C.) and high soil-moisture content, thus confirming the general experience that infection in *rabi jowar*

follows periods of heavy rains and high temperature. The disease is more common in clayey soils than in silt loams.

The morphology and cultural characters of the causative organism have been described.

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REVIEWS

The Cultivated Races of Sorghum. By J. D. Snowden. Printed for the Trustees of the Bentham-Moxon Fund, by Adlard and Son, Ltd., 21, Hart Street, London, W. C. 1, May 1936. Price 10s. 6d.

Spread as sorghum is, over the colonial possessions of many European nations there has been much work of an isolated type done in many places. The latest attempt at a classification of cultivated sorghum was by Dr. Stapf in 1917. He was not in possession of such valuable material as Mr. Snowden was lucky enough to deal with. Mr. Snowden has taken the commendable course of recognising the more distinct types of sorghum as definite units with the status of species. From this point of view he has given a comprehensive classification of cultivated sorghums. Under each group cultural and economic notes have been added. These help the economic botanist to appreciate the status of a variety both in his classification as well as in its economic possibilities. Clear illustrations of typical varieties help in elucidating the description of these species. A final chapter traces the relationships of the wild and cultivated sorghums. An exhaustive bibliography of over 500 references is given. Indices of botanical names, of vernacular names, and of countries and regions complete the helpfulness of this valuable publication. The Trustees of the Bentham-Moxon Fund have to be congratulated for financing the publication of this useful and thorough book. It is up to workers in sorghum in the various parts of the world to utilise this valuable publication and gain help in completing the knowledge on this important food crop. [G. N. R. A.]

Flour Milling Processes. By J. H. Scott. (Published by Messrs. Chapman & Hall, London). Pp. XV + 416, Price 21s. net.

This work by J. H. Scott does not claim any finality in its presentation of the laws governing the processes of flour milling. Prior to the publication of this book, except for "The Physical Science of Flour Milling" by Sir Ernest Simon, there has not existed any work wherein the machinery employed to mill wheat into flour has been taken step by step and its operations reduced to scientific data.

Mr. Scott has done this and also measured the extent of the effect of external influences on the operations and although the result is not perfect it is extremely valuable in itself and also as a very sound foundation, together with the above-mentioned work, for the building up of scientific literature on the subject of flour manufacture.

The need of this has been urgent for many years. Knowledge of the subject has been shrouded in a mass of guess work, confusing because of the many varying factors to be found.

The amount of exact knowledge of wheat and flour, though far from complete, has been greater than that available concerning the operations employed in converting one into the other.

Mr. Scott has gone far towards supplying the deficiency and reasons and presents his conclusions very clearly.

Pioneer work of this nature is difficult and laborious and it may be thought that too little attention has been given to some subjects and too much to others. This is almost inevitable.

Experienced millers will be familiar with the problems discussed but will find old facts presented in a more convincing way and also many new ideas. Knowledge based on hearsay and supposition is ignored or refuted. This, of course, renders the book particularly useful to students.

Especially valuable, I think, are the sections on "Behaviours of moisture in wheat", "Conditioning" and "Machine and commercial flour". All that is written on roll action is extremely interesting and well reasoned but in this the author relies more on theory—very carefully compiled theory.

The effort expended in the preparation of Section V—"The bushel weight of wheat" is to my mind rather wasted. Varying conditions are so numerous as to make this method valueless except as a rough guide.

The rest of the book maintains the high standard the author has set himself, makes very interesting reading and covers all the main mechanical and some of the bio-chemical problems to be met in flour manufacture.

Auxiliary plant and its effect of mill products is not fully treated.

The appendix with which the work concludes is very useful in parts and in parts too complex for the reviewer to offer any opinion. [H. F. M.]

THE DESTRUCTIVE INSECTS AND PESTS ACT, 1914

THE DESTRUCTIVE INSECTS AND PESTS ACT, 1914, AND THE RULES FRAMED THEREUNDER BY THE GOVERNOR-GENERAL-IN-COUNCIL FOR REGULATING THE IMPORT OF PLANTS, ETC., INTO BRITISH INDIA

Corrected up to 1st October 1936

ACT No. II OF 1914

(Amended up to May 1931)

PASSED BY THE GOVERNOR-GENERAL OF INDIA IN COUNCIL

(Received the assent of the Governor-General on the 3rd February 1914)

AN ACT TO PREVENT THE INTRODUCTION INTO BRITISH INDIA OF ANY INSECT, FUNGUS OR OTHER PEST, WHICH IS OR MAY BE DESTRUCTIVE TO CROPS

WHEREAS it is expedient to make provision for preventing the introduction into British India of any insect, fungus or other pest, which is or may be destructive to crops ; it is hereby enacted as follows :—

Short title.

1. This Act may be called the Destructive Insects and Pests Act, 1914.

Definitions.

2. In this Act, unless there is anything repugnant in the subject or context,—

(*a*) " crops " includes all agricultural or horticultural crops, and trees or bushes ;

(*b*) " import " means the bringing or taking by sea, land or air ; and

(*c*) " infection " means infection by any insect, fungus or other pest injurious to a crop.

Power of Governor-General-in-Council to regulate or prohibit the import of articles likely to infect.

3. (1) The Governor-General-in-Council may, by notification in the Gazette of India, prohibit or regulate, subject to such restrictions and conditions as he may impose, the import into British India, or any part thereof, or any specified place therein, of any article or class of articles likely to cause infection to any crop.

(2) A notification under this section may specify any article or class of articles, either generally or in any particular manner, whether with reference to the country of origin, or the route by which imported or otherwise.

Operation of notification under section 3.

4. A notification under section 3 shall operate as if it had been issued under section 19 of the Sea Customs Act, 1878, and the officers of Customs at every port shall have the same powers in respect of any article with regard to the importation of which such a notification has been issued as they have for the time being in respect of any article the importation of which is regulated, restricted or prohibited by the law relating to Sea Customs, and the law for the time being in force relating to Sea Customs or any such article shall apply accordingly. VIII of 1878.

Power of Local Government to make rules.

5. (1) The Local Government may, subject to the control of the Governor-General-in-Council, make rules for the detention, inspection, disinfection or destruction of any article or class of articles in respect of which a notification has been issued under section 3 or of any article which may have been in contact or proximity thereto, and for regulating the powers and duties of the officers whom it may appoint in this behalf.

(2) In making any rule under this section the Local Government may direct that a breach thereof shall be punishable with fine, which may extend to one thousand rupees.

Protection to persons acting under Act.

6. No suit, prosecution or other legal proceeding shall lie against any person for anything in good faith done or intended to be done under this Act.

RULES FOR REGULATING THE IMPORT OF PLANTS, ETC., INTO BRITISH INDIA

Notification No. F.-320/35-A., dated the 20th July 1936

In exercise of the powers conferred by sub-section (1) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), and in supersession of the notification of the Government of India in the late Department of Revenue and Agriculture, No. 580-240, dated the 22nd June 1922, the Governor-General-in-Council is pleased to issue the following order for the purpose of prohibiting, regulating and restricting the import into British India of the articles hereinafter specified.

1. In this order—

(*i*) " Official certificate " means a certificate granted by the proper officer or authority in the country of origin ; and the officers and authorities named in the 3rd column of the first Schedule appended hereto are the proper officers and authorities to grant, in the countries named in the second column, the certificates required by the provisions referred to in the first column thereof ;

(*ii*) " Plant " means a living plant or part thereof but does not include seeds ; and

(*iii*) " Prescribed port " means any of the following ports, namely, Bombay, Calcutta, Cochin, Dhanushkodi, Karachi, Madras, Negapatam, Rangoon and Tuticorin ;

(*iv*) All provisions referring to plants or seeds shall apply also to all packing material used in packing or wrapping such plants or seeds.

2. No plant shall be imported into British India by means of the letter or sample post ; provided that sugarcane for planting intended to be grown under the personal supervision of the Government Sugarcane Expert, Coimbatore, may be imported by him by such post.

3. No plant shall be imported into British India by air ; provided that plants which are infested with living insects and are intended for the introduction of such living insects may be so imported if they are accompanied by a special certificate from the Imperial Entomologist to the Government of India that such plants are imported for the purpose of introducing such insects.

4. No plants other than fruits and vegetables intended for consumption, potatoes and sugarcane shall be imported into British India by sea except after fumigation with hydrocyanic acid gas and at a prescribed port :

Provided that plants which are infested with living parasitized insects and are intended for the introduction of such parasites may be imported without such fumigation if they are accompanied by a special certificate from the Imperial Entomologist to the Government of India that such plants are imported for the purpose of introducing such parasites.

Provided also that in the case of plants imported direct by Mr. M. K. Seetharama Chetty so long as he holds the post of Superintendent, Government Gardens, Mysore, such fumigation shall be dispensed with on condition that Mr. Seetharama Chetty makes himself personally responsible for the effective fumigation under the supervision of the Mycologist of the Mysore Agricultural Department

of all such plants within four days of their landing in India, and that before release from the fumigatorium the plants are certified as free from insect pests and diseases by the Government Mycologist and by the Government Entomologist, Mysore State.

Provided also that rubber plants, stumps and bud wood which the certificate required by paragraph 5 shows to have been grown in Sumatra or in the Federated Malay States and which are intended to be grown in the Tenasserim Division of Burma, may be imported without such fumigation at any port within the Tenasserim Division of Burma.

5. No plants, other than fruits and vegetables intended for consumption and potatoes, shall be imported into British India by sea unless accompanied by an official certificate in the form prescribed in the third schedule that they are free from injurious insects and diseases.

6. Potatoes shall not be imported into British India by sea unless they are accompanied by—

(*a*) a certificate from the consignor stating fully in what country and in what district of such country the potatoes were grown and guaranteeing that warty disease was not known to exist on the farms where the potatoes were grown ; and

(*b*) an official certificate that no case of warty disease of potatoes has been known during the twelve months preceding the date of the certificate within five miles of the place where the potatoes were grown.

Provided that potatoes may be imported from Italy if they are accompanied by a certificate of freedom from disease granted by a Royal Phytopathological Institute in Italy.

Provided also that potatoes may be imported under a certificate from the Imperial Economic Botanist, Imperial Agricultural Research Institute, New Delhi.

7. Rubber plants shall not be imported into British India by sea unless, in addition to the general certificate required under Rule 5 they are accompanied by an official certificate that the estate from which the plants have originated or the individual plants are free from *Fomes semitostus, Sphœrostible repens, Fusicladium macrosporum* and *Oidium heveæ.*

8. No Lemon plants, Lime plants, Orange plants, Grape-Fruit plants or other citrus plants and no cuttings of such plants shall be imported into British India unless, in addition to the general certificate required under Rule 5 they are

accompanied by an official certificate that they are free from the *Mal de Secco Deuterophoma tracheiphila* or that the disease does not exist in the country in which they were grown.

9. (1) The importation of sugarcane into British India by sea from the Fiji Islands, New Guinea, Australia or the Philippine Islands is prohibited absolutely.

(2) The importation of sugarcane into British India by sea from any other country is prohibited unless, in addition to the general certificate required under Rule 5 it is accompanied by an official certificate that it has been examined and found free from cane borers, scale insects, aleurodes, root disease (any form), pineapple disease (*Thielaviopsis paradoxa*), sereh and cane gummosis, that it was obtained from a crop which was free from mosaic disease and that the Fiji disease of sugarcane does not occur in the country of export:

Provided that in the case of canes for planting imported direct by the Government Sugarcane Expert, Coimbatore, and intended to be grown under his personal supervision, such certificates shall be required only in respect of the freedom of the country of export from the Fiji disease of sugarcane.

10. Hevea rubber plants and Hevea rubber seeds shall not be imported into British India from America or from the West Indies except by the Director of Agriculture, Madras Presidency.

11. (*a*) Seeds of flax, berseem and cotton shall not be imported by letter or sample post, otherwise than by sea.

(*b*) The importation of 'Mexican Jumping Beans' (*Sebastiania palmeri* of the family Euphorbiaceæ) is prohibited absolutely.

12. Coffee plants, coffee seeds and coffee beans shall not be imported into British India except for experimental planting purposes only by the Director of Agriculture, Madras Presidency, who shall take all measures necessary to ensure that such coffee plants, beans or seeds as are imported by him are free from plant diseases and injurious insects. Provided that the prohibition hereinbefore contained shall not apply (*i*) to roasted and ground coffee, or (*ii*) to a consignment of unroasted or unground coffee beans or seeds produced in India and covered by a certificate of origin in the form set forth in the fourth schedule appended hereto and signed by one of the authorities specified in the fifth schedule appended hereto.

13. Flax seeds and berseem (Egyptian clover) seeds shall not be imported into British India by sea, unless the consignee produces before the Collector of Customs a license from a Department of Agriculture in India in that behalf.

14. (1) Unginned cotton other than cotton from a Kathiawar port which has been produced in India shall not be imported by sea or by air.

(2) Cotton seed shall not be imported by sea save for experimental purposes by one of the officers named in the second schedule appended hereto, and shall not be so imported by such officer save at the port of Bombay and in quantities not exceeding one hundredweight in any one consignment and on condition that it will be fumigated on importation with carbon bisulphide :

Provided that, if the cotton seed is accompanied by a certificate from a Government Entomologist of the country of origin to the effect that the seed and its container have been treated in such a way as to destroy all insect life, the seed shall be examined on importation by such officer as the Governor-General-in-Council may appoint and shall not be required to be refumigated unless such examination shows that refumigation is necessary.

15. Nothing in these rules shall be deemed to apply to—

(1) the import of any article into Aden, or

(2) the bringing by sea or by air from one port or place in British India, not being in Aden, to another such port or place.

FIRST SCHEDULE [*Paragraph 1 (i)*]

Paragraph	Country of origin	Authority
1	2	3
6 (*b*)	Great Britain and Ireland .	The Ministry of Agriculture and Fisheries, England. The Department of Agriculture, Scotland. The Ministry of Agriculture, Northern Ireland. The Department of Agriculture and Technical Instruction for Ireland.
	Sweden	The Ministry of Agriculture.
	Norway	The Norwegian Board of Agriculture.
	Denmark	The Ministry of Agriculture.
	France.	Ditto.
	Japan (including Formosa) .	The Ministry of Agriculture and Forestry.
	Italy	The Ministry of Agriculture.
	Kenya Colony . . .	The Department of Agriculture.
	Australia	Chief Quarantine Officer for Plants.
	New Zealand . . .	Department of Agriculture, Industries and Commerce, Wellington.
	Holland	The Department of Agriculture.
	Germany	Ditto.
	Hungary	Royal Hungarian Service for Protection of Plants (M. Kir Novenyvedelmi Szolgalat).

Paragraph	Country of origin	Authority
1	2	3
	* Other countries . . .	The Ministry or Department of Agriculture of the country concerned.
7	Ceylon	The Department of Agriculture.
	Malay Peninsula . . .	The Department of Agriculture, Straits Settlements and Federated Malay States.
	Dutch Indies . . .	The Department of Agriculture, Industry and Commerce.
	Belgian Congo . . .	Ditto.
	Kenya Colony . . .	The Department of Agriculture.
	Uganda Protectorate . .	Ditto.
	Nyassaland	Ditto.
	South Africa . . .	The Union of South Africa, Department of Agriculture.
	Japan (including Formosa) .	The Ministry of Agriculture and Forestry.
8	Italy	The Ministry of Agriculture, Royal Italian Phytopathological Institute.
	Greece	Ministry of Agriculture.
	Palestine	Department of Agriculture and Forests.
	South Africa . . .	The Union of South Africa, Department of Agriculture.
	Northern Rhodesia . .	Department of Agriculture.
	Southern Rhodesia . .	Ditto.
	Other countries . . .	The Ministry or Department of Agriculture of the country concerned.
9	Dutch Indies . . .	The Department of Agriculture, Industry and Commerce.
	Mauritius	The Department of Agriculture.
	Philippine Islands . .	The Bureau of Agriculture.
	Japan (including Formosa) .	The Ministry of Agriculture and Forestry.
	South Africa . . .	The Union of South Africa, Department of Agriculture.
	Egypt	The Ministry of Agriculture.
	West Indies	The Imperial College of Tropical Agriculture, St. Augustine, Trinidad.
	Windward and Leeward Islands	Advisory Department of Agriculture at the Imperial College.
	British Guiana . . .	The Department of Science and Agriculture.
	Trinidad	The Department of Agriculture.
	Jamaica	Ditto.
	United States . . .	Ditto.
	Ceylon	Ditto.
	Malay Peninsula . . .	The Department of Agriculture, Straits Settlements and Federated Malay States.
	Kenya Colony . . .	The Department of Agriculture.
	Queensland	The Department of Agriculture and Stock.

* When a Customs officer receives a certificate required by clause (*b*) of rule 6 from any country not specified by name in the part of the schedule which relates to such certificate, he shall after passing the consignment forward the certificate to the Government of India, Education, Health and Lands Department, for information.

SECOND SCHEDULE [*Paragraph 14 (2)*]

The Director, Imperial Institute of Agricultural Research, New Delhi.
The Secretary, Indian Central Cotton Committee, Bombay.
The Director of Agriculture, Madras Presidency, Madras.
The Director of Agriculture, Bombay Presidency, Poona.
The Chief Agricultural Officer in Sind, Karachi.
The Director of Agriculture, Punjab, Lahore.
The Director of Agriculture, United Provinces, Lucknow.
The Director of Agriculture, Central Provinces, Nagpur.
The Director of Agriculture, Bengal, Dacca.
The Director of Agriculture, Bihar, Sabour.
The Director of Agriculture, Assam, Shillong.
The Director of Agriculture, Burma, Rangoon.
The Agricultural Officer, North-West Frontier Province, Peshawar.
The Director of the Institute of Plant Industry, Indore.
The Director of Agriculture, His Exalted Highness the Nizam's Dominions, Hyderabad.
The Director of Agriculture, Mysore State, Bangalore.
The Director of Agriculture, Baroda State, Baroda.
The Director of Agriculture, Gwalior State, Gwalior.
The Director of Agriculture and Fisheries, Travancore State, Trivandrum.
The Superintendent of Agriculture and Panchayats, Cochin State, Cochin.
The Revenue Commissioner, Porbandar State, Porbandar.
The Chief Revenue Officer, Nawanagar State, Nawanagar.
The Agricultural Research Officer, Nawanagar State, Nawanagar.
The Revenue Commissioner, Bhavnagar State, Bhavnagar.
The Revenue Commissioner, Morvi State, Morvi.
The Revenue Commissioner, Junagadh State, Junagadh.
The President of the Chamber of Agriculture, Pondicherry.

THIRD SCHEDULE (*Paragraph 5*)

Certificate

This is to certify that the plant(s), living plant(s) or plant products (*Strike out the words not applicable*) included in the consignment, of which particulars are given below were/was thoroughly examined on the (date)..... by (name).....a duly authorised official of the..... .....and found to be healthy, no evidence of the presence of any injurious insect, pest, or disease [destructive to agricultural or horticultural crops or to trees or bushes] having been found in/on them and that the consignment (including the packing) covered by this certificate has/has not been treated in the

following manner (*e.g.*, fumigated with.....or disinfected with.....)
prior / immediately subsequent to inspection.

Inspected / Not inspected in the field by a duly authorised inspector on.....

(Signature).....

(Official status).....

Date.....

No. and description of packages.....

Distinguishing marks.....

Description of plants or plant products or parts thereof.....

Stated to be grown at.....

Exported by.....

Name and address of consignee.....

Name of vessel or particulars of route.....

Date of shipment.....

Port or place of entry.....

Additional Certificate(s) attached.....

(Give here details of any special certificate or certificates issued in respect of imports specifically scheduled).

FOURTH SCHEDULE (*Paragraph 12*)

Certificate of origin for Indian coffee beans or seeds

Name of consignor	Name of consignee	Gross weight	Number of packages	Mark of each package	Name of district in which produced

Certified that the above consignment of raw coffee beans or seeds is Indian produce grown in the district specified above.

Signature of certifying authority.

No. of Railway Receipt or
No. of Bill of Lading.

Signature of Consignor.

FIFTH SCHEDULE (*Paragraph 12*)

Certifying authority :—

(*i*) The Assistant Director of Agriculture, Chickmagalur, Kadur District, Mysore State.

(*ii*) The Director of Agriculture and Fisheries, Travancore State.

(*iii*) The Director of Agriculture, Cochin Government.

(*iv*) The Director of Agriculture, Madras Presidency.

(*v*) Any other officer whom the Government of Madras may appoint in this behalf by notification in the Fort St. George Gazette.

Notification No. 1581-Agri., dated the 1st October 1931

In exercise of the powers conferred by Sub-section (1) of Section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), hereinafter referred to as the said Act, and in supersession of the notifications of the Government of India in the Department of Education, Health and Lands, Nos. 1493, dated the 14th November 1925 and 1561, dated the 26th November 1925, and of all notifications amending the same, the Governor-General-in-Council is pleased to issue the following order for the purpose of regulating the import into British India of American cotton :—

1. In this order :—

(*i*) "Cotton" includes ginned cotton, and droppings strippings, fly and other waste products of a cotton mill other than yarn waste, but does not include cotton seed or unginned cotton.

(*ii*) "American Cotton" means all cotton produced in any part of America.

(*iii*) "Bale" means any pressed package of cotton of whatever size or density.

(*iv*) "Square Bale" means a bale of a weight exceeding 270 lbs.

(*v*) "Round Bale" means a bale of a weight not exceeding 270 lbs.

2. American cotton shall not be imported into British India by means of the letter or sample post or as passengers' accompanied baggage and shall not be imported by any other means save through the port of Bombay and subject to the following conditions :—

(*a*) On or before the departure of a ship carrying a consignment of American cotton for Bombay from the port from which the cotton is consigned, the consignee shall ascertain the name of the ship, the

probable date of its arrival in Bombay, and the number of square and round bales of American cotton contained in the consignment, and shall furnish this information to the Docks Manager of the Bombay Port Trust not less than 14 days before the arrival of the ship at Bombay: provided that where the cotton is loaded for Bombay at Port Said or at a European port, the ordinary length of voyage from which is less than three weeks, it shall be sufficient to furnish the information not less than ten days before the arrival of the ship at Bombay;

(*b*) On arrival at Bombay, the cotton shall be disinfected in such manner as shall be prescribed in rules made by the Government of Bombay under section 5 of the said Act;

(*c*) Prior to taking delivery of the cotton the importer shall pay a sum at the rate hereinafter provided, which shall cover the cost of fumigation, including the cost of loading the cotton into the barge, conveyance to the fumigation wharf or bunder, unloading from the barge after fumigation and delivery at the bunder, but not including docks import charges as specified in the Bombay Port Trust Scale of Rates charged at the docks:

Provided that samples of American cotton imported by parcel post or as ship's parcels, not exceeding 20 lbs. each in weight shall be fumigated free of cost in the fumigation Chamber of the Bombay Customs House;

Provided further that the Docks Manager of the Bombay Port Trust may, in accordance with any general or special orders of the Governor-General-in-Council for the time being in force, remit or refund, in whole or in part, any sum which under this order is payable or has been paid on any consignment of American cotton;

(*d*) No vessel shall discharge American cotton during a period of rain, mist or drizzle.

3. The rate referred to in clause (*c*) of paragraph 2 shall be Rs. 2-7-0 per square bale and Rs. 1-8-0 per round bale, or when cotton is landed at the special American Cotton wharf in the Bombay Docks Rs. 3-12-0 per square bale and Rs. 2-8-0 per round bale:

Provided as follows:—

(*i*) in cases in which the importer has failed to furnish information in accordance with clause (*a*) of paragraph 2 each of the rates hereinbefore prescribed shall be increased by Rs. 2 per bale;

(*ii*) the minimum fee for the fumigation of any cotton imported at any one time on a single ship shall be rupees one hundred and fifty, and when such minimum fee is leviable, it shall, if there is more than one consignee, be distributed among the consignees in proportion to the amount of their respective consignments.

As amended by Government of India, Education, Health and Lands Department, Notification No. F. 398/33-A., dated 2nd April 1934 ; Notification No. F. 136/34, dated the 17th May 1934 ; Notification No. F. 190/33-A., dated the 13th August 1934 ; Notification No. 4573/34-A., dated the 24th January 1935 and Notification No. F. 34-3/34-A., dated the 4th February 1935.

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

1. Agriculture and Live-stock in India

A bi-monthly Journal of Agriculture and Animal Husbandry for the general reader interested in Agriculture or Live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s*. 9*d*. per annum inclusive of Indian postage. Price per part Rs. 2 or 3*s*. 6*d*. inclusive of Indian postage). Volumes I to VI complete are available.

2. The Indian Journal of Agricultural Science

A bi-monthly Scientific Journal of Agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931. Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s*. per annum inclusive of Indian postage. Price per part Rs. 3 or 5*s*. 3*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

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5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

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ERRATA

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Page 590, line 15, *for* ' apetite ' *read* ' appetite '.
Page 598, line 16 from the bottom, *for* ' 6th July 1932 ' *read* ' 6th December 1932 '.
Page 616, line 2 from the bottom, *for* ' number ' *read* ' numbers '.
Page 634, line 10, *for* ' trocolia ' *read* ' trochela '.
Page 643, line 16, *for* ' bread ' *read* ' bred '.
Page 648, line 16 from the bottom, *for* ' arejet ' *read* ' are jet '.
Page 667, line 14 from the bottom, *for* ' soili n ' *read* ' soil in '.

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